PROTEIN METABOLISM DISORDERS

The Chemist's View of Proteins

- Proteins are made from 20 different amino acids, 9 of which are essential.
- Each amino acid has an amino group, an acid group, a hydrogen atom, and a side group.
- It is the side group that makes each amino acid unique.
- The sequence of amino acids in each protein determines its unique shape and function.

How Does the Body Use Protein?

- Functions of protein
  - Provide structural and mechanical support
  - Maintain body tissues
  - Functions as enzymes and hormones
  - Help maintain acid base balance
  - Transport nutrients
  - Assist the immune system
  - Serve as a source of energy when necessary

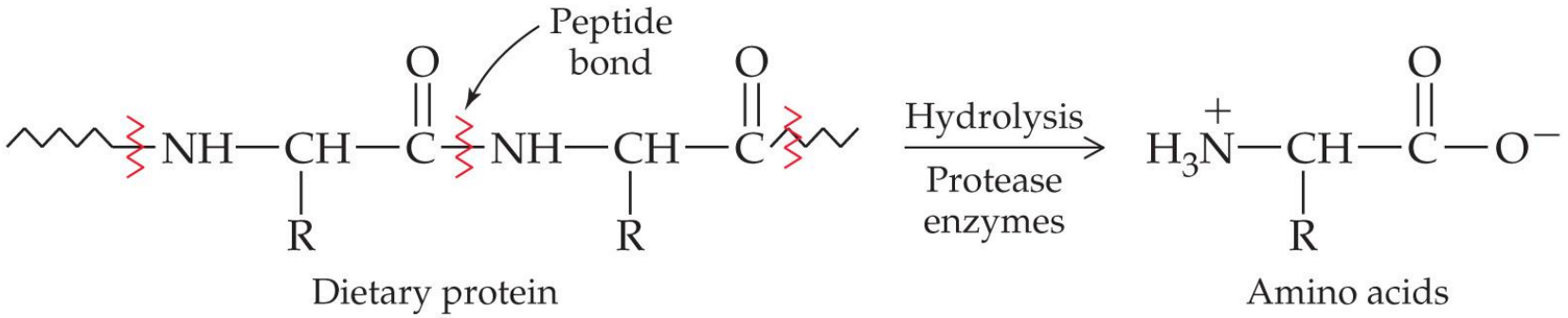
Proteins in the Body

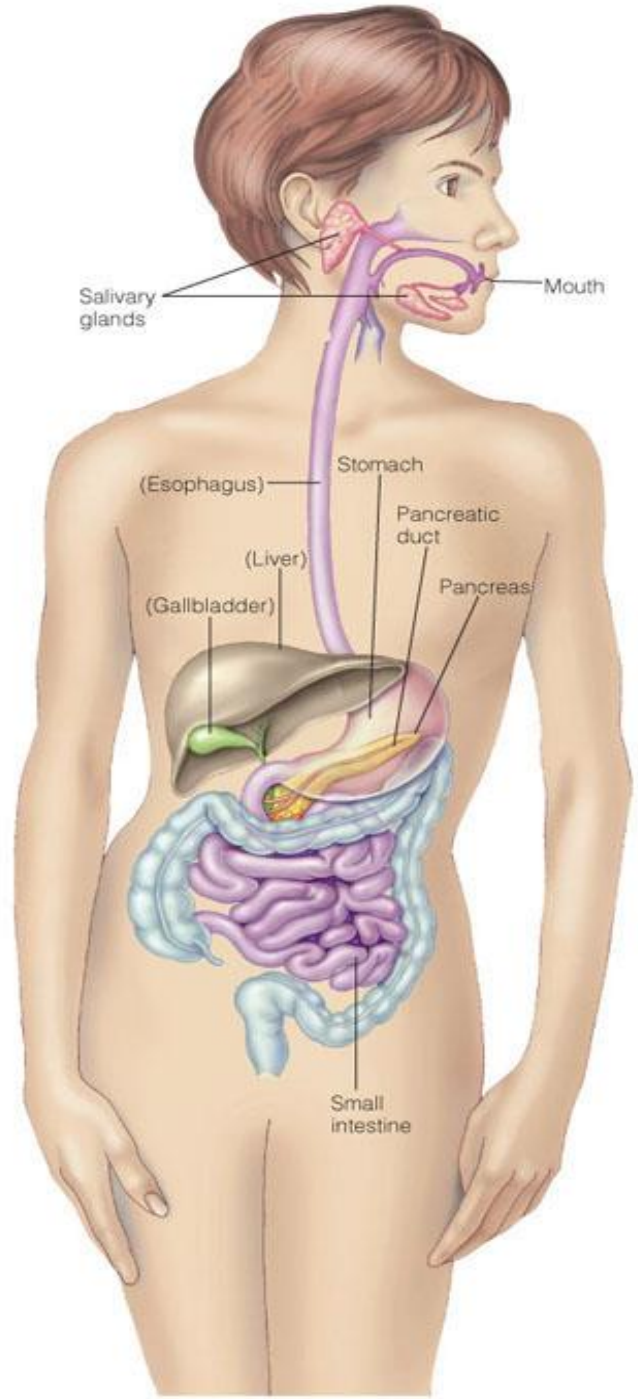
- The synthesis of protein is determined by genetic information.
- Protein is constantly being broken down and synthesized in the body.
- Researchers measure nitrogen balance to study synthesis, degradation and excretion of protein.
- Protein has many important functions in the body.
- Protein can be used for energy if needed; its excesses are stored as fat.
- The study of proteins is called proteomics.

Digestion of Protein

- The goal of protein digestion is the hydrolysis of all peptide bonds to produce free amino acids.
- No chemical digestion of protein occurs in the mouth.

Hydrolysis of peptide bonds *(peptide bonds are amide bonds)*





PROTEIN

Mouth and salivary glands

Chewing and crushing moisten protein-rich foods and mix them with saliva to be swallowed

Stomach

Hydrochloric acid (HCl) uncoils protein strands and activates stomach enzymes:

Protein —(pepsin, HCl)→ smaller polypeptides

Small intestine and pancreas

Pancreatic and small intestinal enzymes split polypeptides further:

Polypeptides —(pancreatic and intestinal proteases)→ tripeptides, dipeptides, amino acids

Then enzymes on the surface of the small intestinal cells hydrolyze these peptides and the cells absorb them:

Peptides —(intestinal tripeptidases and dipeptidases)→ amino acids (absorbed)

HYDROCHLORIC ACID AND THE DIGESTIVE ENZYMES

In the stomach:

Hydrochloric acid (HCl)
- Denatures protein structure
- Activates pepsinogen to pepsin

Pepsin
- Cleaves proteins to smaller polypeptides and some free amino acids
- Inhibits pepsinogen synthesis

In the small intestine:

Enteropeptidase[a]
- Converts pancreatic trypsinogen to trypsin

Trypsin
- Inhibits trypsinogen synthesis
- Cleaves peptide bonds next to the amino acids lysine and arginine
- Converts pancreatic procarboxypeptidases to carboxypeptidases
- Converts pancreatic chymotrypsinogen to chymotrypsin

Chymotrypsin
- Cleaves peptide bonds next to the amino acids phenylalanine, tyrosine, tryptophan, methionine, asparagine, and histidine

Carboxypeptidases
- Cleave amino acids from the acid (carboxyl) ends of polypeptides

Elastase and collagenase
- Cleave polypeptides into smaller polypeptides and tripeptides

Intestinal tripeptidases
- Cleave tripeptides to dipeptides and amino acids

Intestinal dipeptidases
- Cleave dipeptides to amino acids

Intestinal aminopeptidases
- Cleave amino acids from the amino ends of small polypeptides (oligopeptides)

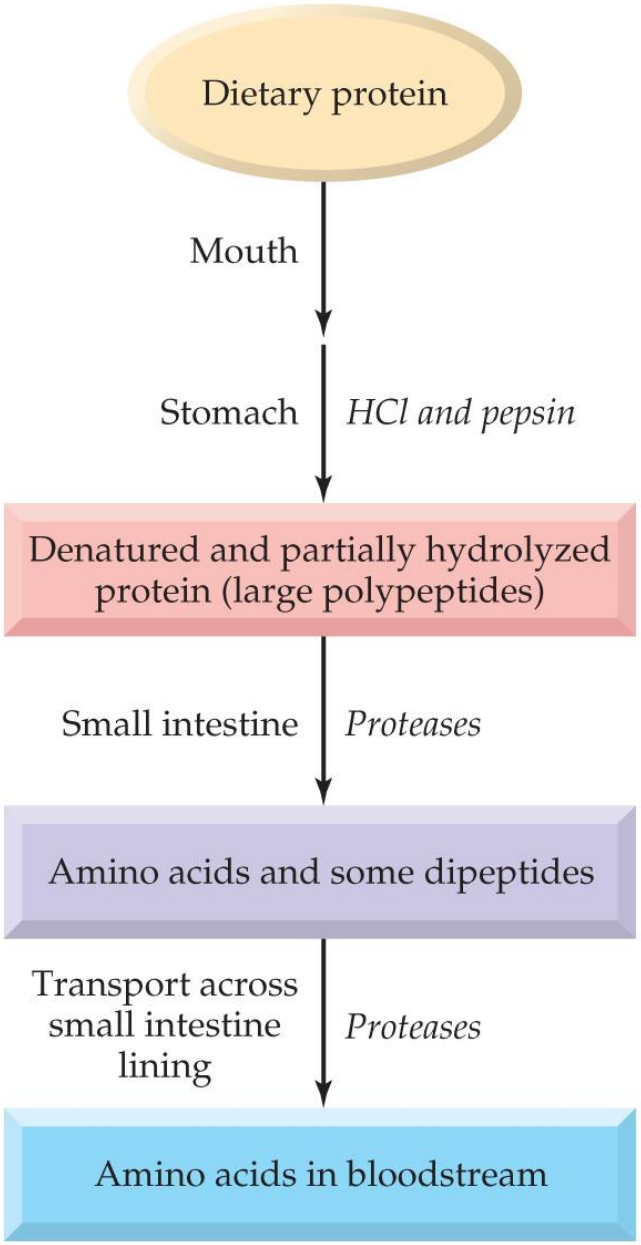
[a]Enteropeptidase was formerly known as *enterokinase*.

Digestion of Proteins (contd.)

HCl in the stomach (pH 1–2) denatures dietary protein.

Gastric secretions also include pepsinogen, which activated by acid produces the enzyme pepsin.

Pepsin is stable and active at pH 1–2, it hydrolyzes some of the peptide bonds in the denatured proteins, which are broken down into smaller polypeptides.



Digestion of Proteins (contd.)

- Next, the polypeptides enter the small intestine, where the pH is about 7–8.
- Proteases such as (trypsin, chymotrypsin, and carboxypeptidase) take over further hydrolysis of peptide bonds in the partially digested proteins.
- The combined action of the pancreatic proteases in the small intestine and other proteases in the cells of the intestinal lining frees the amino acids from dietary proteins.
- After active transport across cell membranes lining the intestine, the amino acids are absorbed directly into the bloodstream.

Digestion of Proteins

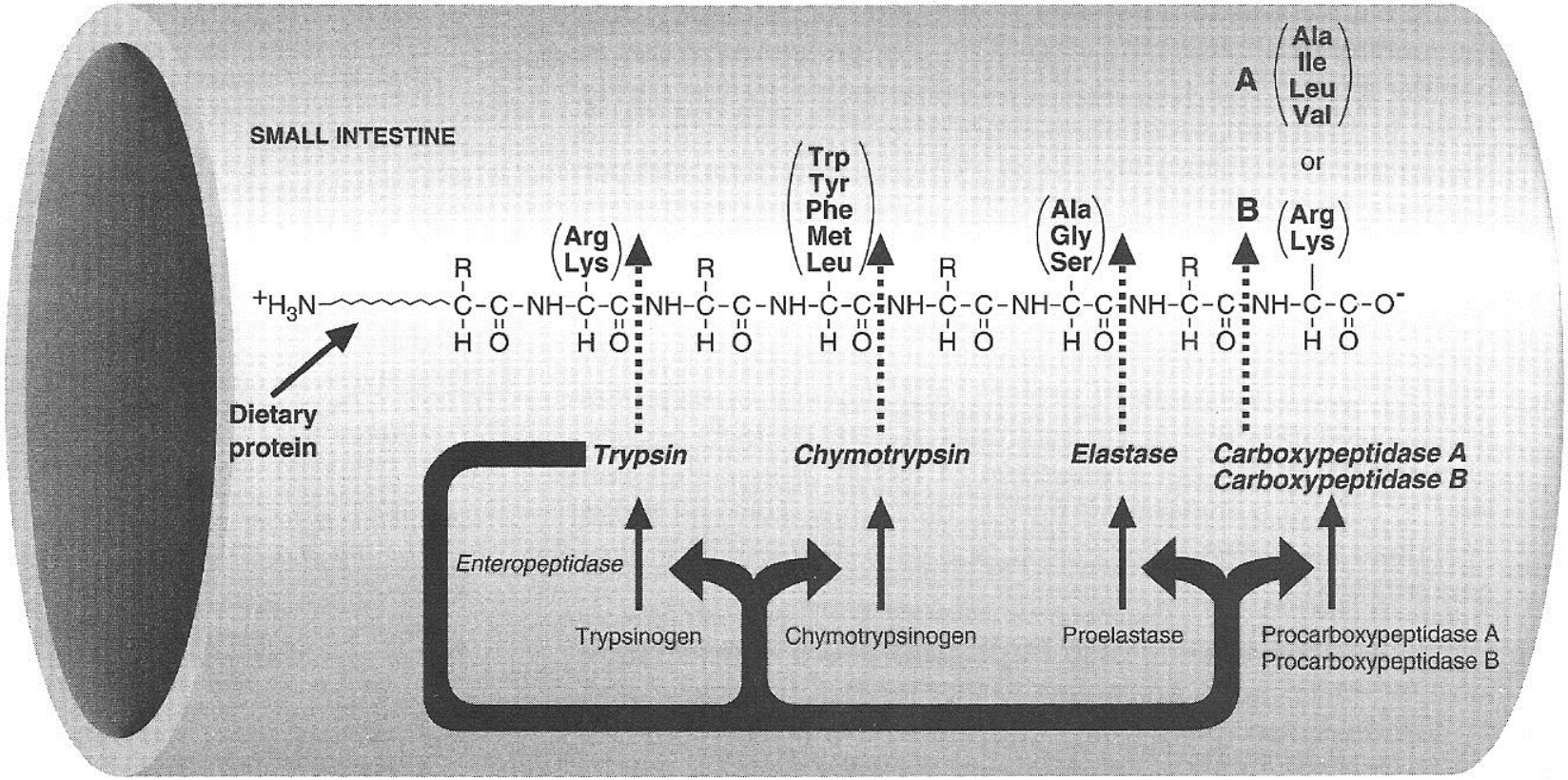


Figure 21.4
Cleavage of dietary protein by proteases from the pancreas. The peptide bonds susceptible to hydrolysis are shown for each of the five major pancreatic proteases. [Note: *Enteropeptidase* is synthesized in the intestine.]

Amino acid pool: The entire collection of free amino acids in the body

Each of the 20 amino acids is degraded via its own unique pathway.

But the general scheme for amino acid catabolism is the same for each one:

-Removal of the amino group

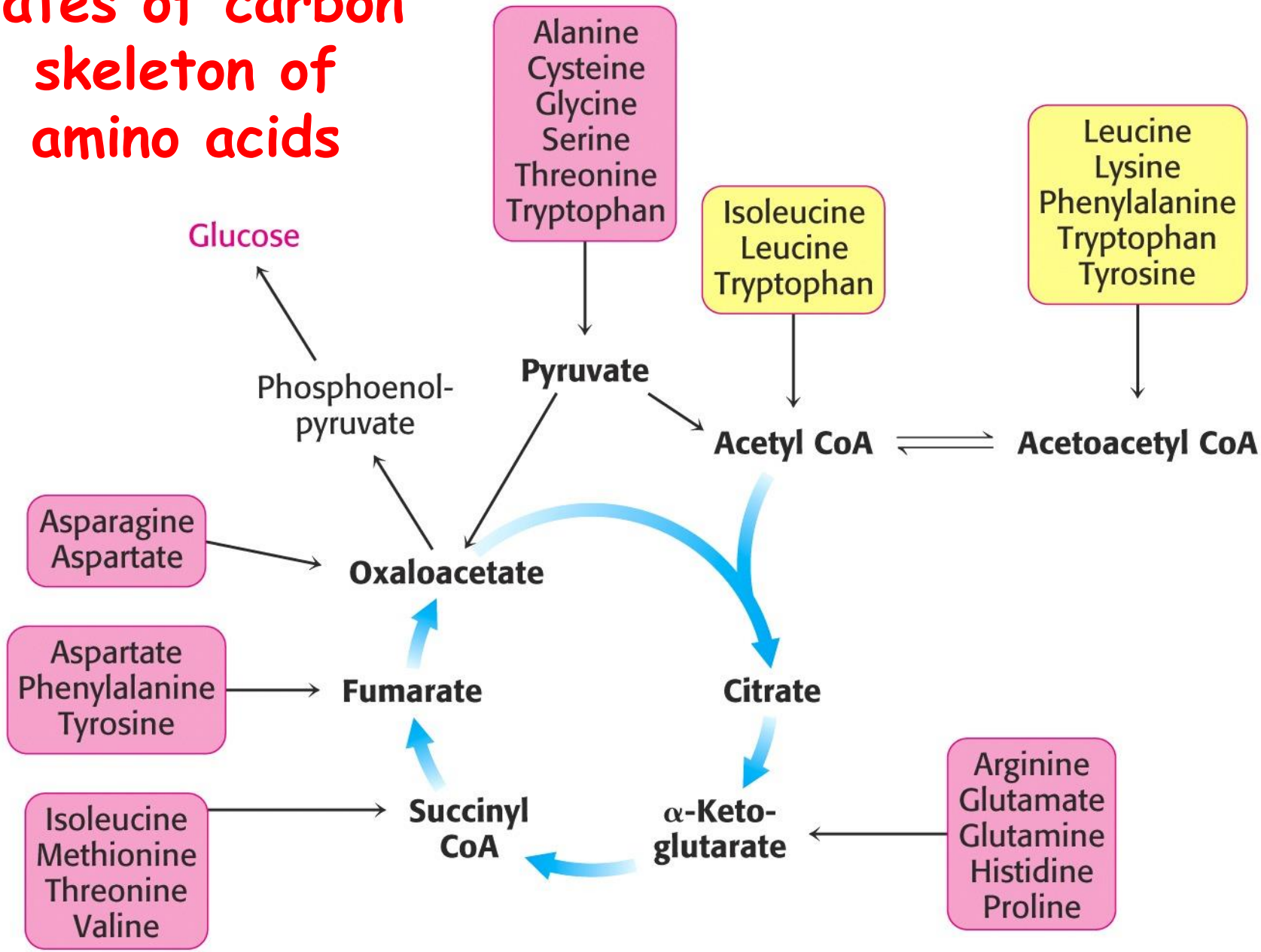
-Use of nitrogen in synthesis of new nitrogen compounds

-Passage of nitrogen into the urea cycle

-Incorporation of the carbon atoms into compounds that can enter the citric acid cycle

Our bodies do not store nitrogen-containing compounds and ammonia is toxic to cells.

Fates of carbon skeleton of amino acids



Ketogenic/Glucogenic Amino Acids

Amino acids converted to acetoacetyl -SCoA Or acetyl-SCoA are called ketogenic amino acids.

Amino acids that proceed by way of oxaloacetate pathway are known as glucogenic amino acids

TABLE 28.1 Glucogenic and Ketogenic Amino Acids

Glucogenic	
Alanine	Glycine
Arginine	Histidine
Aparagine	Methionine
Aspartate	Proline
Cysteine	Serine
Glutamate	Threonine
Glutamine	Valine
Both glucogenic and ketogenic	
Isoleucine	
Lysine	
Phenylalanine	
Tryptophan	
Tyrosine	
Ketogenic	
Leucine	

So….

If the amino acids are not being used to make new proteins, their carbon skeletons can be used to generate energy.

Fate of Amino Nitrogen's

Amino nitrogen are either incorporated into urea and excreted

or be used in the synthesis of new nitrogen-containing compounds such as:

- -Nitric oxide
- -Hormones
- -Neurotransmitters
- -Nicotinamide (in NAD^+ and $NADP^+$)
- -Heme (in red blood cells)
- -Purine and pyrimidine bases (for nucleic acids)

How Much Protein Do You Need?

- Healthy, nonpregnant adults
  - Should consume enough to replace what is used every day
  - The goal is nitrogen balance
- Pregnant woman, people recovering from surgery or injury, and growing children
  - Should consume enough to build new tissue

Nitrogen Balance and Imbalance

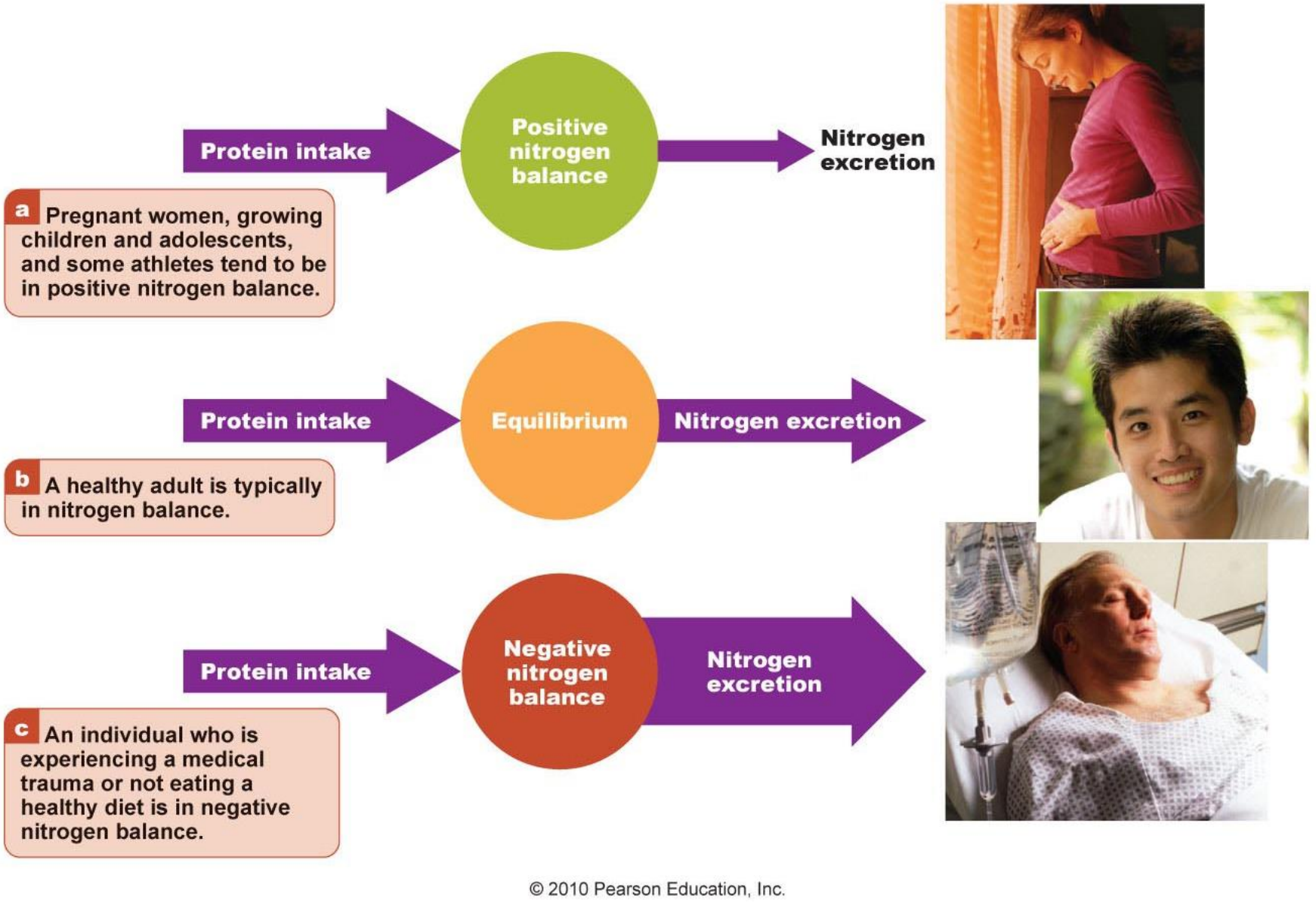


Figure 6.12

Eating Too Little Protein

- Protein-energy malnutrition (PEM)
  - Protein is used for energy rather than its other functions in the body
  - Other important nutrients are in short supply
  - More prevalent in infants and children
    - 17,000 children die each day as a result

Too Little Protein

- Without adequate protein
  - Cells lining the GI tract are not sufficiently replaced as they slough off
  - Digestive function is inhibited
  - Absorption of food is reduced
  - Intestinal bacteria gets into the blood and causes septicemia
  - Immune system is compromised due to malnutrition and cannot fight infection

Types of PEM: Kwashiorkor

- Severe protein deficiency
  - Generally result of a diet high in grains and deficient in protein
- Symptoms range from
  - Edema in legs, feet, and stomach
  - Muscle tone and strength diminish
  - Hair is brittle and easy to pull out
  - Appear pale, sad, and apathetic
  - Prone to infection, rapid heart rate, excess fluid in lungs, pneumonia, septicemia, and water and electrolyte imbalances

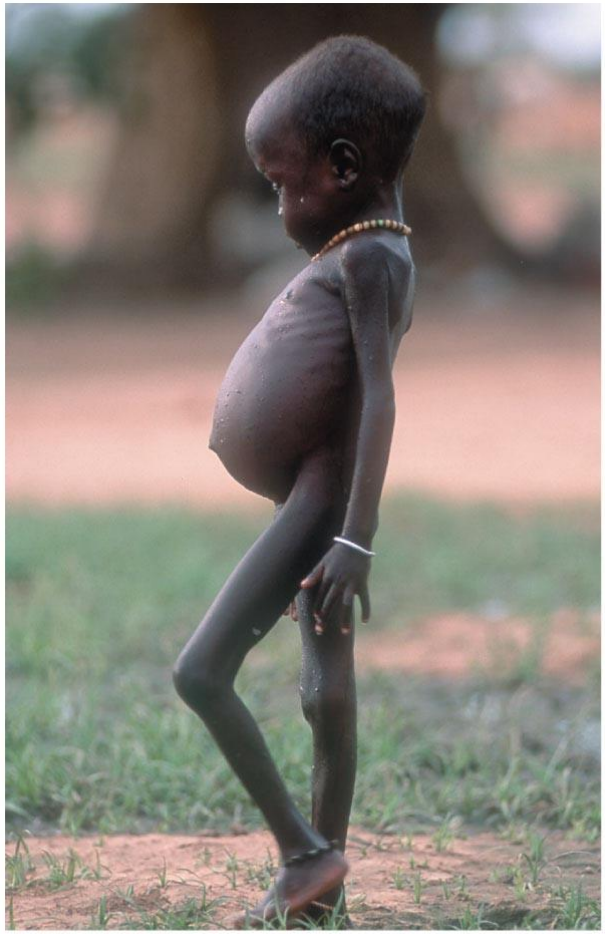
Figure 6.16

Types of PEM: Marasmus

- Results from a severe deficiency in kilocalories
  - Frail, emaciated appearance
  - Weakened and appear apathetic
  - Many cannot stand without support
  - Look old
  - Hair is thin, dry, and lacks sheen
  - Body temperature and blood pressure are low
  - Prone to dehydration,infections, and unnecessary blood clotting

Types of PEM: Marasmic Kwashiorkor

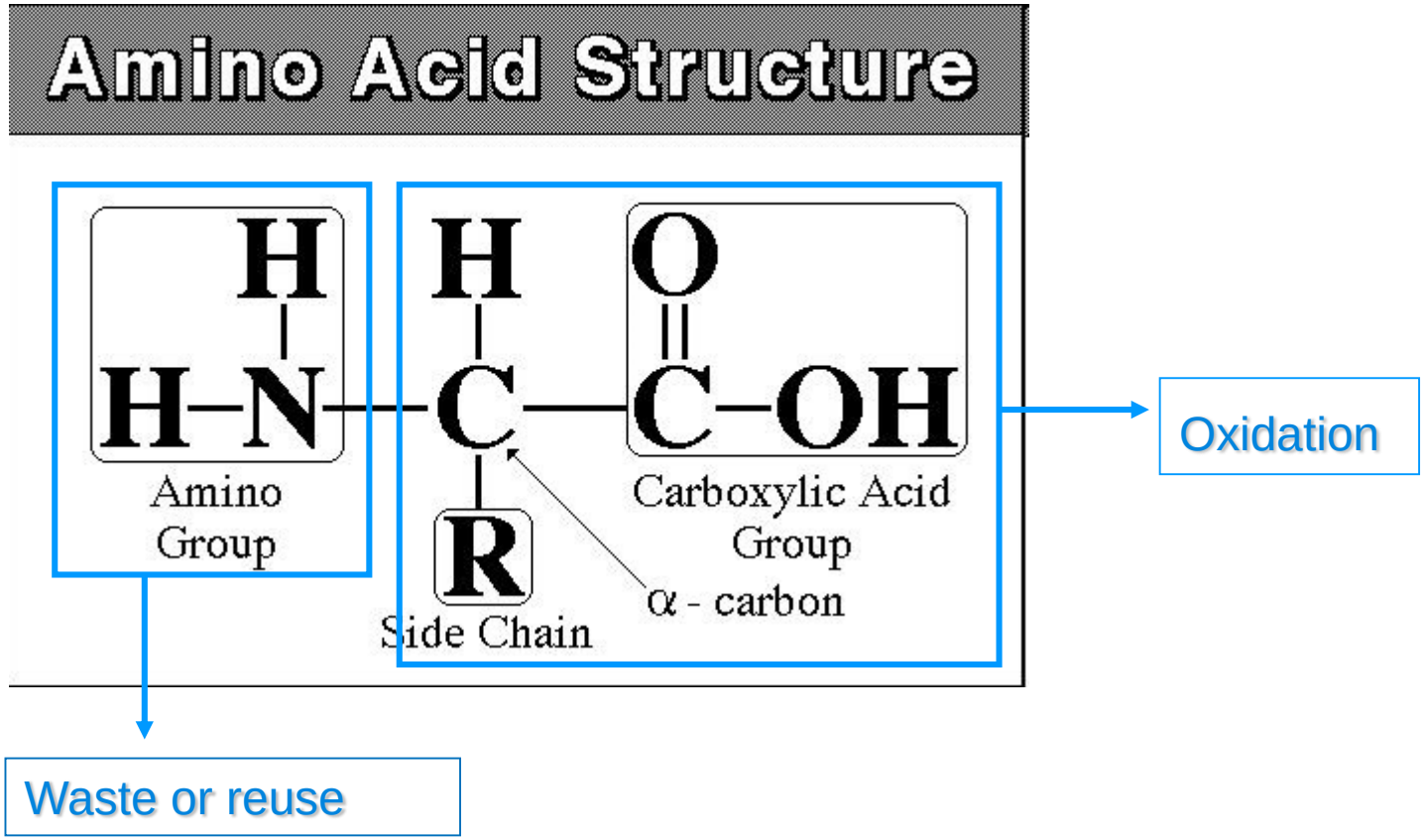
- Chronic deficiency in kilocalories and protein
  - Have edema in legs and arms
  - Have a “skin and bones” appearance
  - With treatment the edema subsides and appearance becomes more like someone with marasmus



Health Effects and Recommended Intakes of Protein

- Health Effects of Protein
  - Heart Disease
    - Foods high in animal protein also tend to be high in saturated fat.
    - Homocysteine levels increase cardiac risks.
  - Cancer
    - A high intake of animal protein is associated with some cancers.
    - Is the problem high protein intake or high fat intake?
  - Adult Bone Loss (Osteoporosis)
    - High protein intake associated with increased calcium excretion.
    - Inadequate protein intake affects bone health also.

Amino acid oxidation and the production of urea



Ammonia has to be eliminated

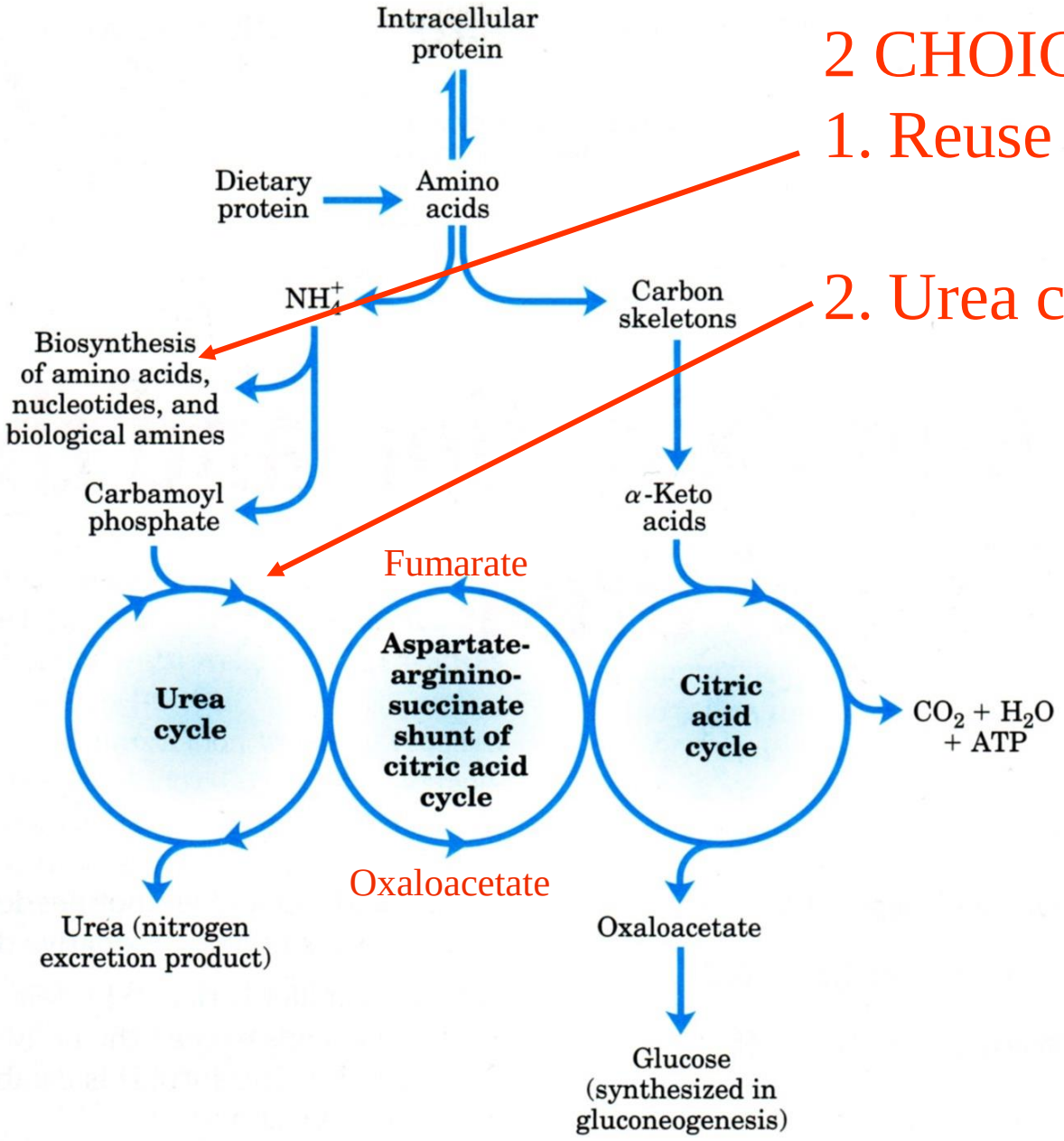
- Ammonia originates in the catabolism of amino acids that are primarily produced by the degradation of proteins – dietary as well as existing within the cell:
  - digestive enzymes
  - muscle proteins
  - hemoglobin
  - intracellular proteins (damaged, unnecessary)

Ammonia has to be eliminated

- Ammonia is toxic, especially for the CNS, because it reacts with α-ketoglutarate, thus making it limiting for the TCA cycle ⇒ decrease in the ATP level

- Liver damage or metabolic disorders associated with elevated ammonia can lead to tremor, slurred speech, blurred vision, coma, and death

- Normal conc. of ammonia in blood: 30-60 μM



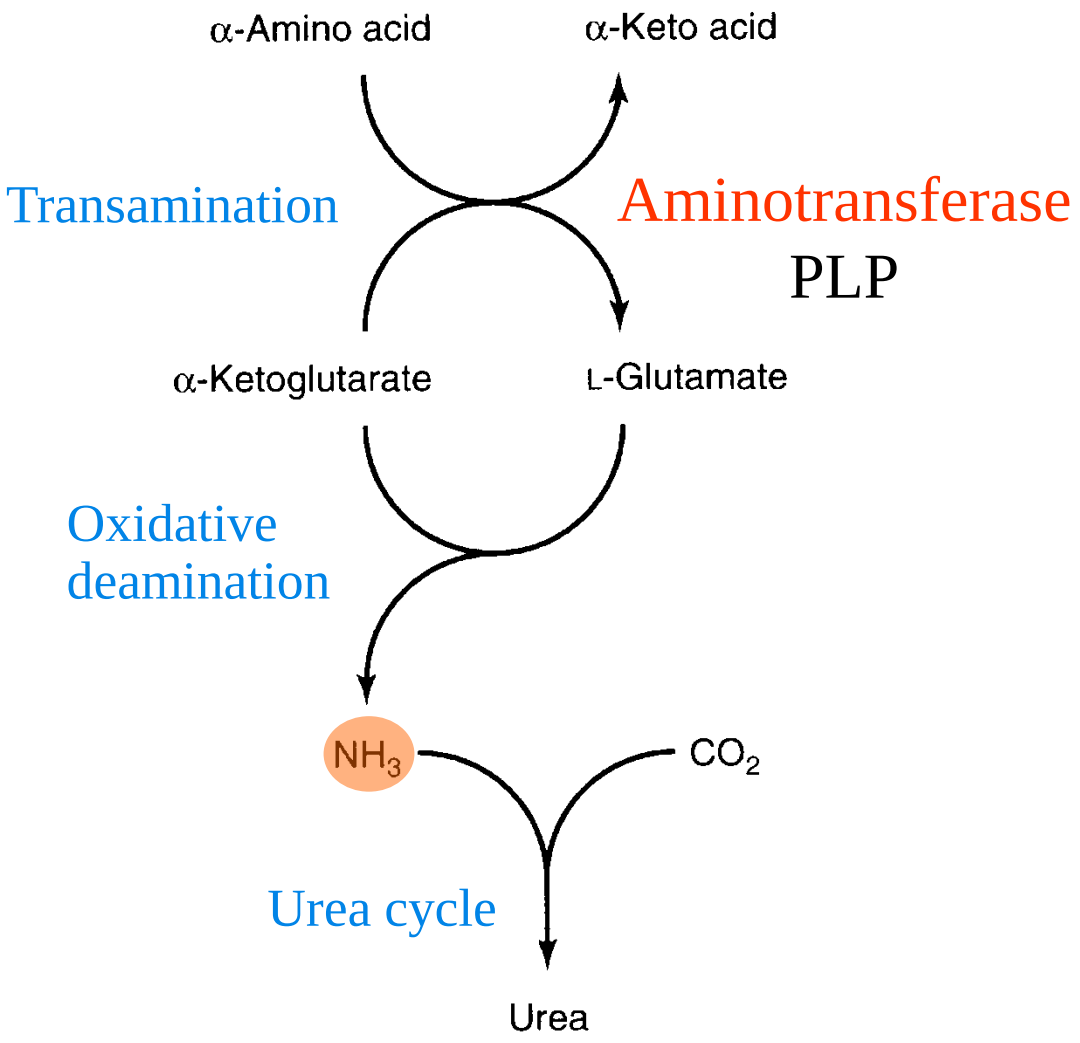
2 CHOICES

1. Reuse

2. Urea cycle

Overview of amino acid catabolism in mammals

Nitrogen removal from amino acids



Nitrogen removal from amino acids

Step 1: Remove amino group

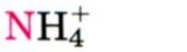
Step 2: Take amino group to liver for nitrogen excretion

Step 3: Entry into mitochondria

Step 4: Prepare nitrogen to enter urea cycle

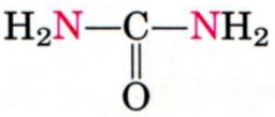
Step 5: Urea cycle

Excretory forms of nitrogen



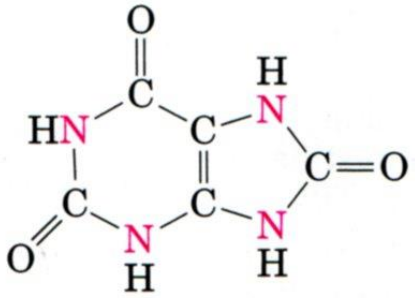
Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia



Urea

Ureotelic animals: many terrestrial vertebrates; also sharks



Uric acid

Uricotelic animals: birds, reptiles

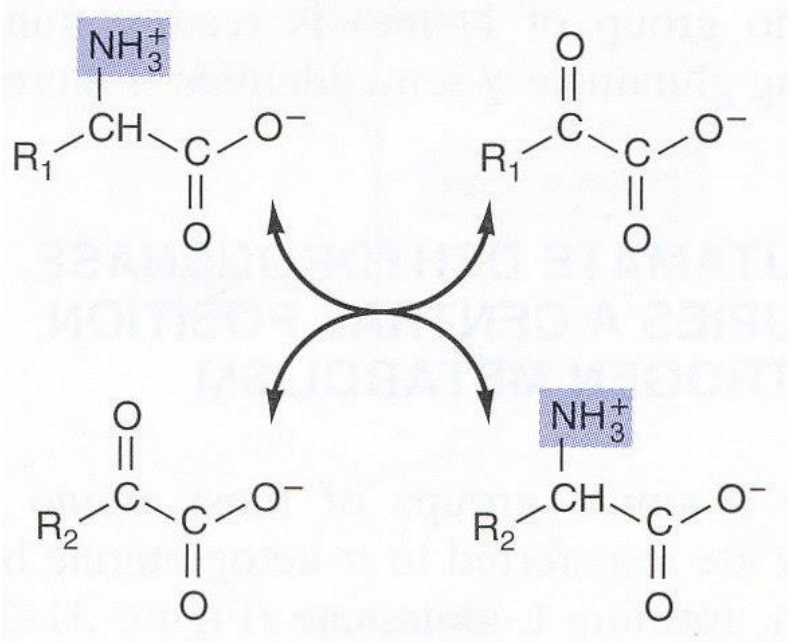
a) Excess NH_4^+ is excreted as ammonia (microbes, aquatic vertebrates or larvae of amphibia),

b) Urea (many terrestrial vertebrates)

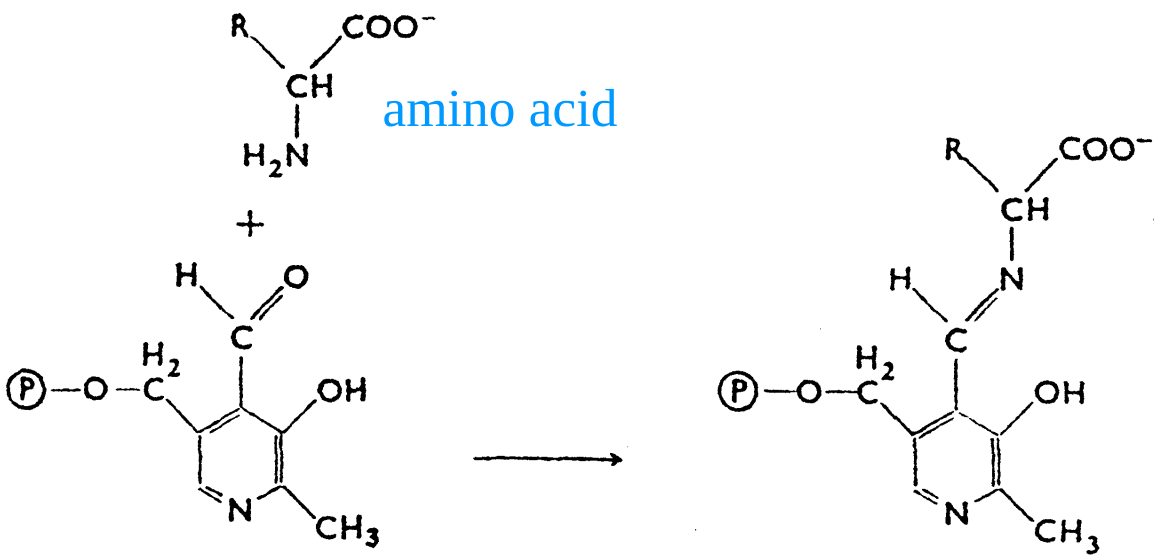
c) or uric acid (birds and terrestrial reptiles)

Step 1. Remove amino group

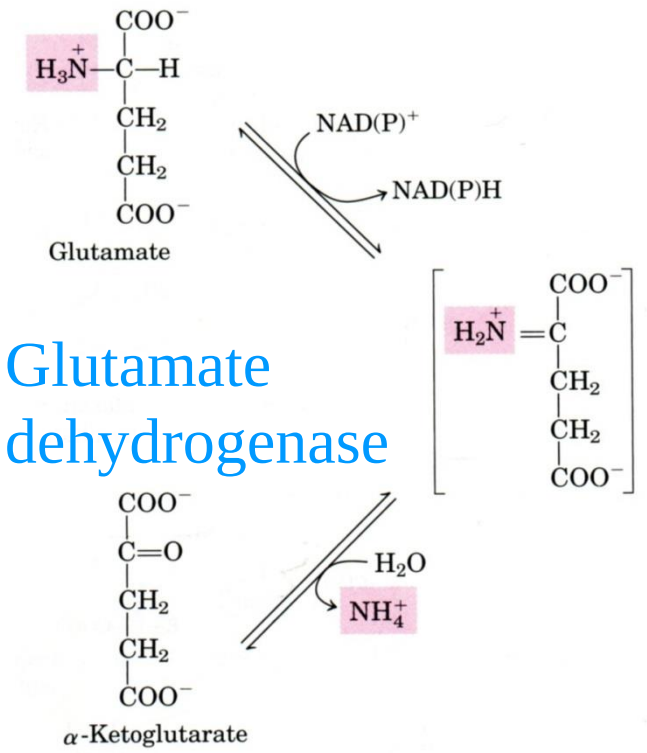
- Transfer of the amino group of an amino acid to an α-keto acid ⇒ the original AA is converted to the corresponding α-keto acid and vice versa:



- Transamination is catalyzed by transaminases (aminotransferases) that require participation of pyridoxalphosphate:



Step 2: Take amino group to liver for nitrogen excretion



Glutamate releases its amino group as ammonia in the liver.

The amino groups from many of the α-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules.

The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor

Nitrogen carriers

1. Glutamate

   transferres one amino group WITHIN cells:

   Aminotransferase → makes glutamate from α-ketogluta-rate

   Glutamate dehydrogenase → opposite

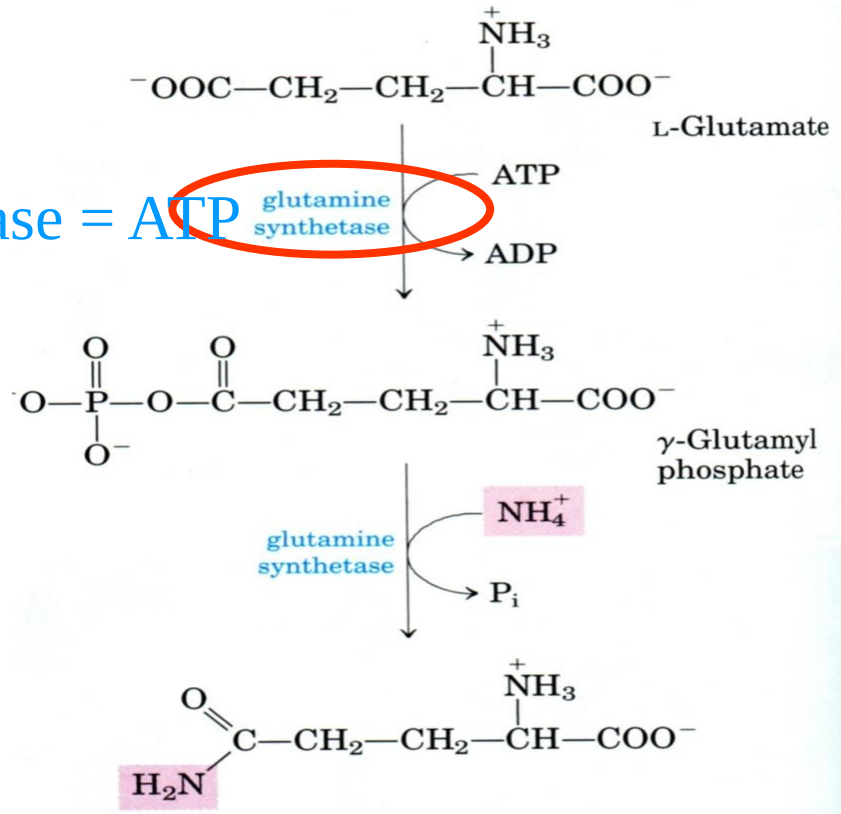
2. Glutamine

   transferres two amino group BETWEEN cells → releases its amino group in the liver

3. Alanine

   transferres amino group from tissue (muscle) into the liver

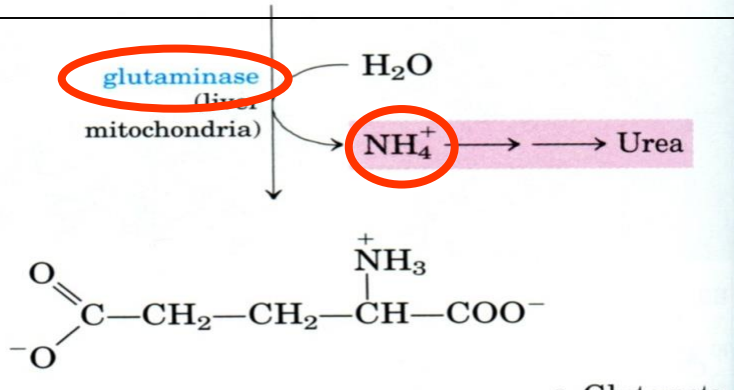
SynthAtase = ATP



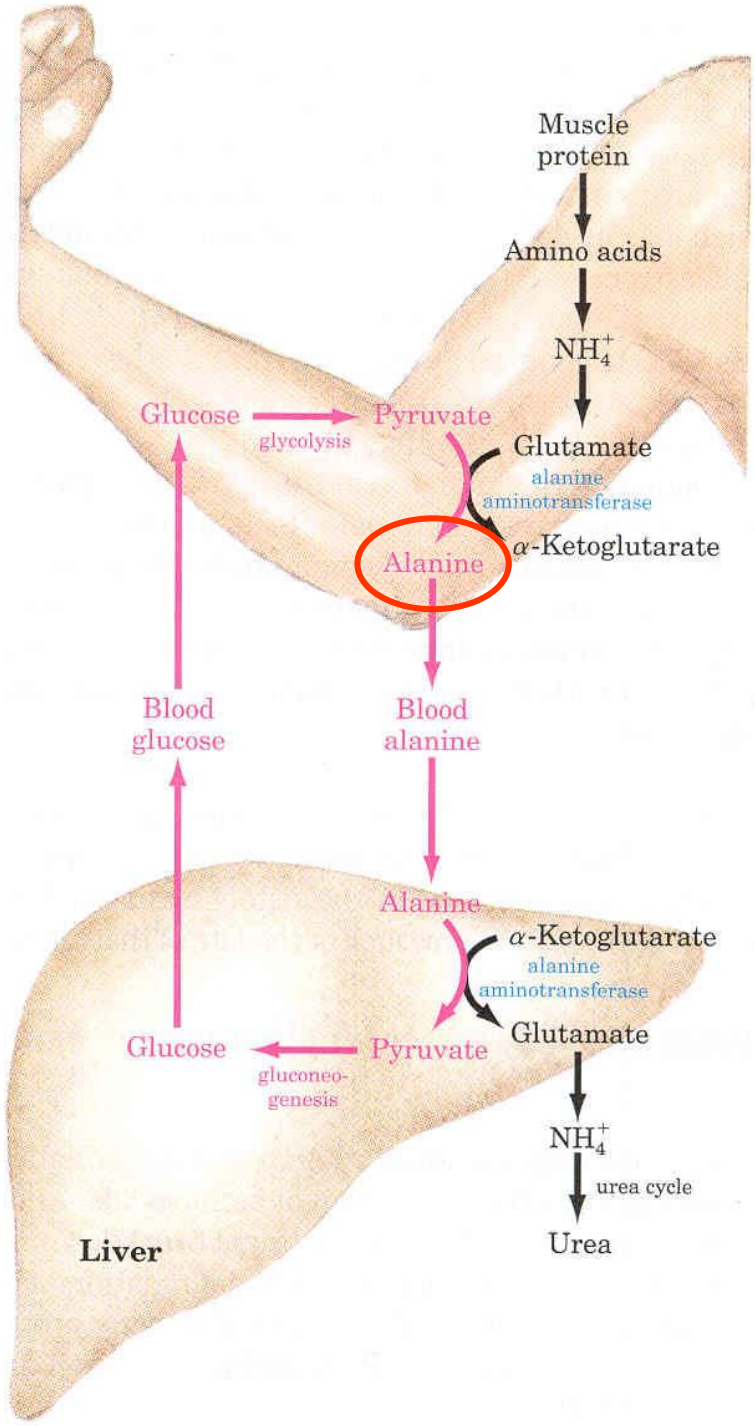
Move within cells

Move between cells

In liver



Glucose-alanine cycle



Alanine plays a special role in transporting amino groups to liver.

Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.
The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

Sources of ammonia for the urea cycle:

- Oxidative deamination of Glu, accumulated in the liver by the action of transaminases and glutaminase
- Glutaminase reaction releases NH_3 that enters the urea cycle in the liver (in the kidney, it is excreted into the urine)
- Catabolism of Ser, Thr, and His (nonoxidative deamination) also releases ammonia:

Serine - threonine dehydratase

$$\text{Serine} \rightarrow\rightarrow \text{pyruvate} + NH_4^+$$

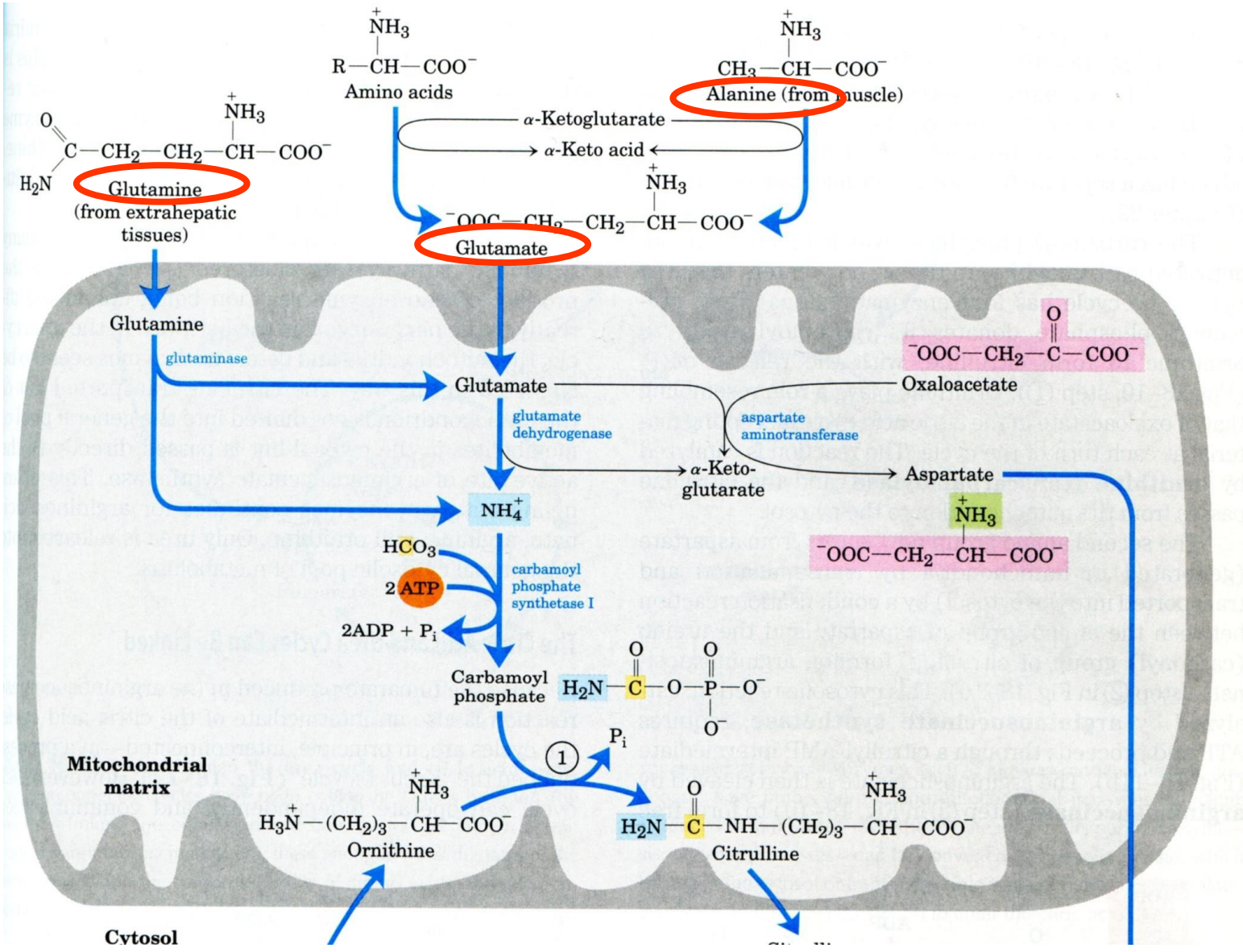
$$\text{Threonine} \rightarrow\rightarrow \alpha\text{-ketobutyrate} + NH_4^+$$

- Bacteria in the gut also produce ammonia.

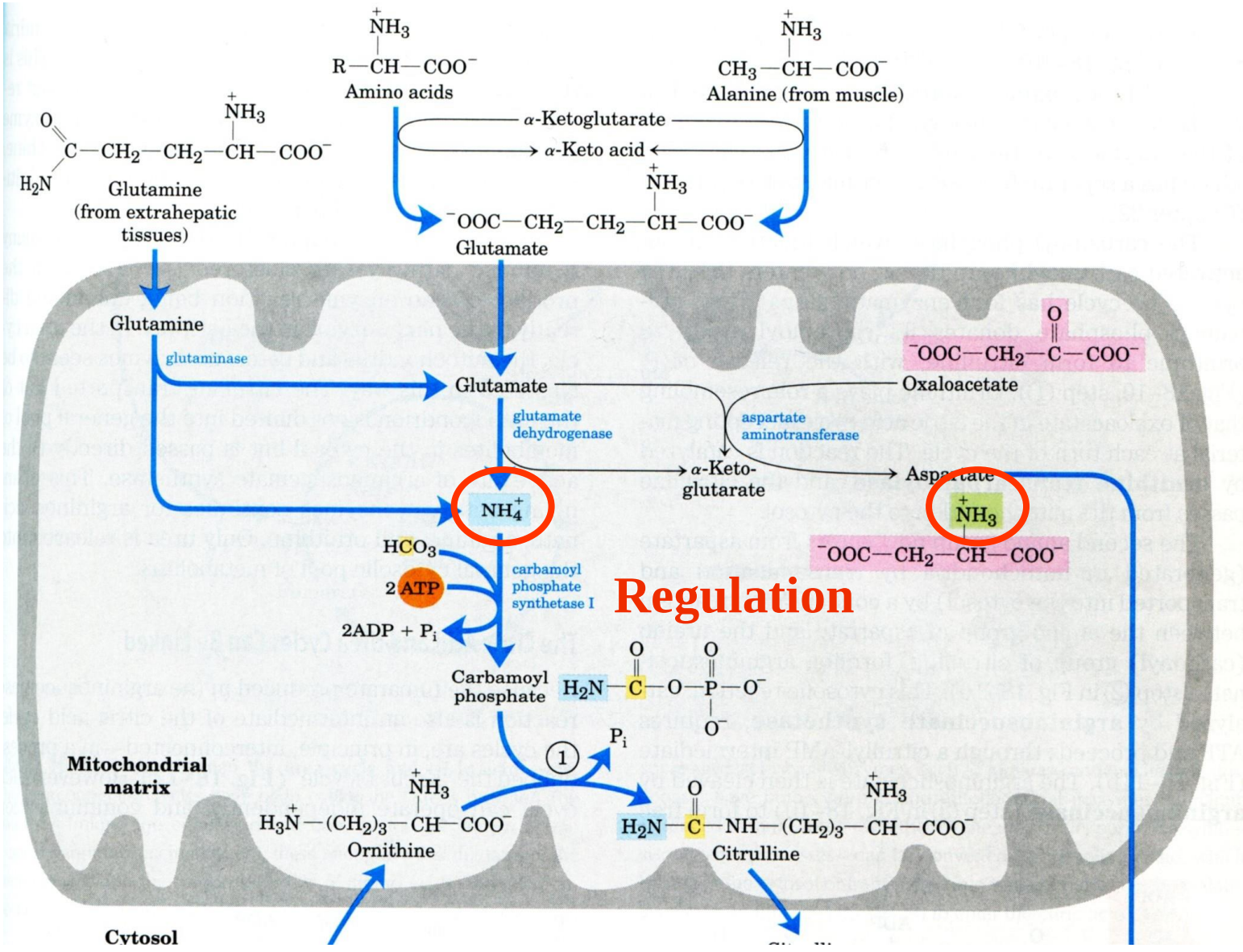
Review:

- Nitrogen carriers ⇨ glutamate, glutamine, alanine
- 2 enzymes outside liver, 2 enzymes inside liver:
  - Aminotransferase (PLP) → α-ketoglutarate → glutamate
  - Glutamate dehydrogenase (no PLP) → glutamate → α-ketoglutarate (**in liver**)
  - Glutamine synthase → glutamate → glutamine
  - Glutaminase → glutamine → glutamate (**in liver**)

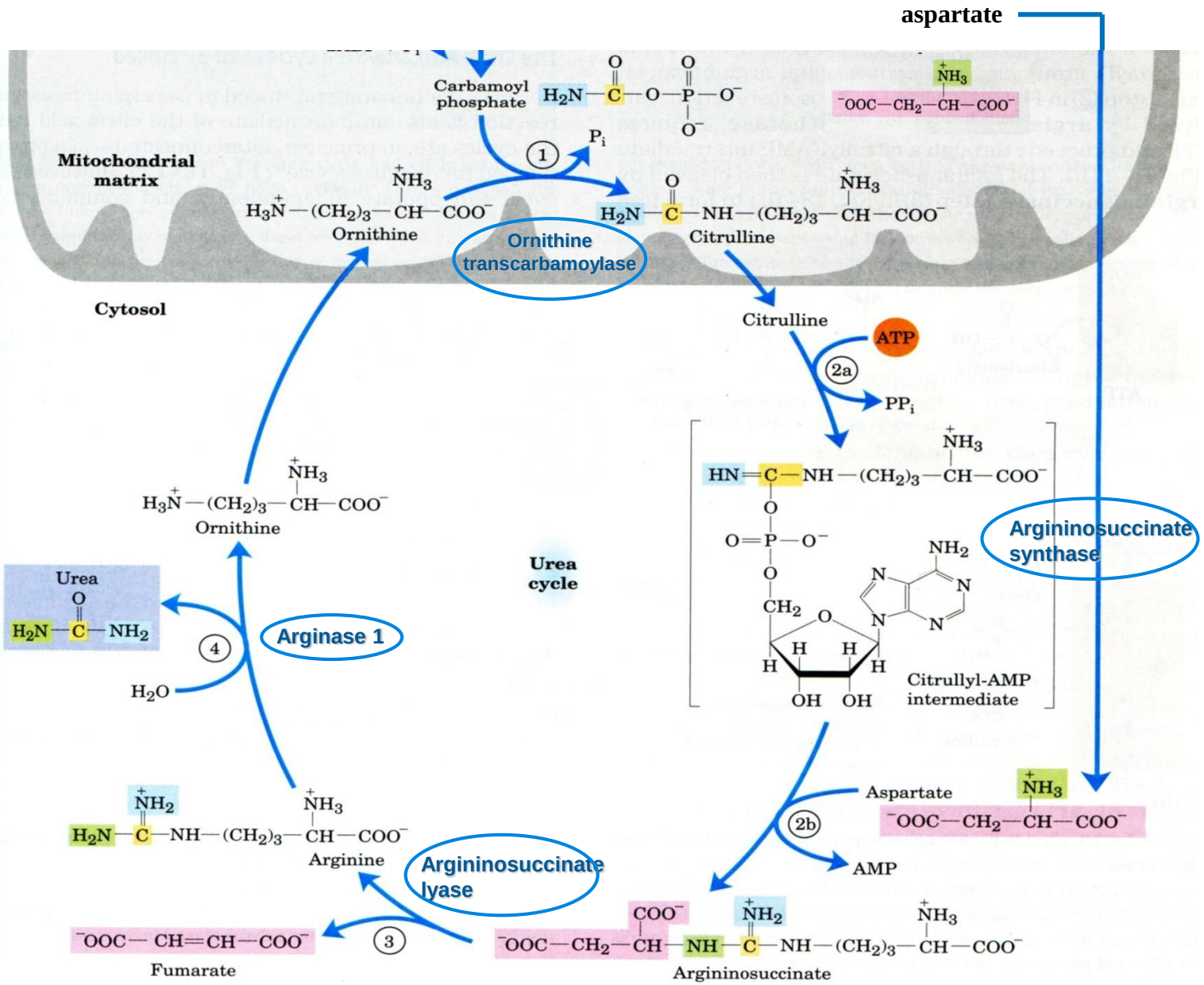
Step 3: entry of nitrogen to mitochondria



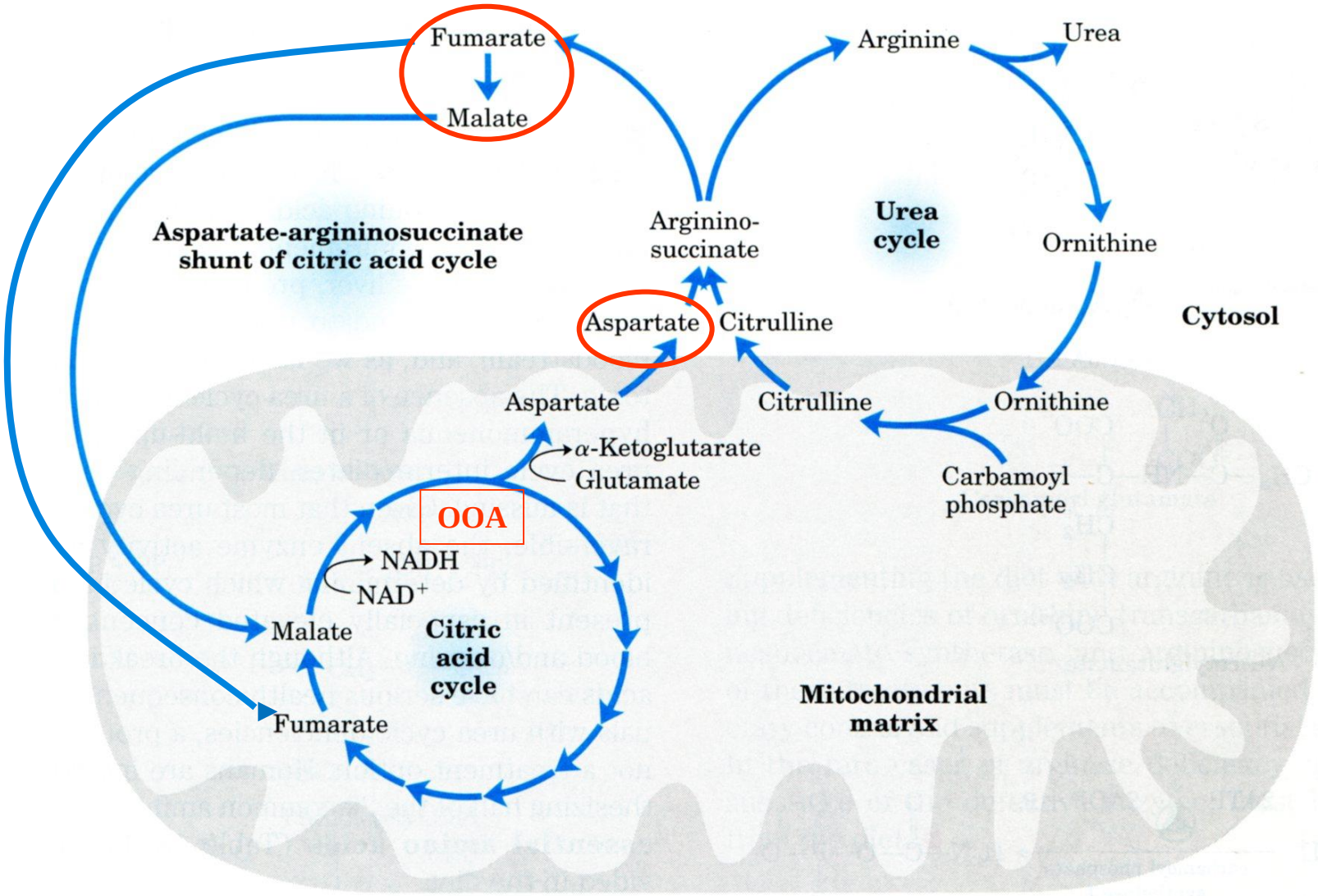
Step 4: prepare nitrogen to enter urea cycle



Step 5: Urea cycle



Oxaloacetate → aspartate



Urea cycle – review
(Sequence of reactions)

- ***Carbamoyl phosphate*** formation in mitochondria is a prerequisite for the urea cycle
  - (*Carbamoyl phosphate synthetase*)
- ***Citrulline*** formation from carbamoyl phosphate and ornithine
  - (*Ornithine transcarbamoylase*)
- Aspartate provides the additional nitrogen to form ***argininosuccinate*** in cytosol
  - (*Argininosuccinate synthase*)
- ***Arginine*** and ***fumarate*** formation
  - *(Argininosuccinate lyase*)
- Hydrolysis of arginine to ***urea*** and ornithine
  - (*Arginase*)

Deficiencies of urea cycle enzymes

Ammonia toxicity

Ammonia encephalopathy

- Increased concentration of ammonia in the blood and other biological fluids → ammonia difuses into cells, across blood/brain barrier → increased synthesis of glutamate from α-ketoglutarate, increased synthesis of glutamine
- α-ketoglutarate is depleted from CNS → inhibition of TCA cycle and production of ATP
- Neurotransmitters – glutamate (excitatory neurotr.) and GABA (inhibitory neurotr.), may contribute to the CNS effects – bizarre behaviour

Deficiencies of urea cycle enzymes

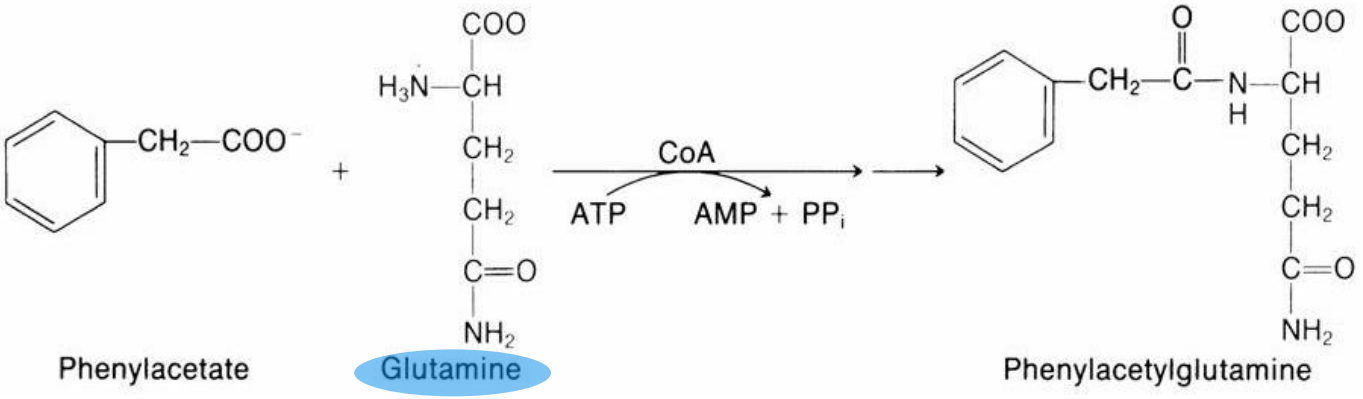
- Infant born with total deficiency of one or more enzymes survive at least several days.
- Many enzymes deficiencies are partial → enzymes have altered K_m values.
- Case are known of deficiencies of each enzymes.
- Interruption of the cycle at each point affected nitrogen metabolism differently - some of the intermediates can diffuse from hepatocytes → accumulate in the blood → pass into the urine.
- If symptoms are not detected early enough → severe mental retardation → **brain damage is irreversible.**

N-acetylglutamate synthase deficiency:

- Deficiency or genetic mutation of enzyme (autosomal recessive) → urea cycle failure.
- A severe neonatal disorder with fatal consequences, if not detected immediately upon birth.
- Hyperammonemia and general hyperaminoacidemia in a newborn (liver contain no detectable ability to synthesize N-acetylglutamate).
- Early symptoms include lethargy, vomiting, and deep coma.
- ***Treatment*** with structural analog N-carbamoyl-L-glutamate – activates CPS-I, mitigates the intensity of the disorder,

Carbamoyl phosphate synthetase (CPS I) deficiency:

- autosomal recessive metabolic disorder, associated with mental retardation and developmental delay.
- Hyperammonemia has been observed in 0 – 50% of normal level of CPS-I synthesis in the liver.
- Treatment with *benzoate* and *phenylacetate* ⟶ hippurate and Phe-Ac-Gln are excreted in the urine:



Ornithine transcarbamoylase (OTC) deficiency

- The most common urea cycle disorder, resulting in a mutated and ineffective form of the enzyme.
- X-linked recessive disorder caused by a number of different mutations in the OTC gene – males are generally more seriously affected than females (males are asymptomatic as heterozygotes).
- Complications with OTC may include mental retardation and developmental delay.

Argininosuccinate synthase deficiency – citrullinemia (citrullinuria)

- autosomal recessive metabolic disorder, inability to condense citrulline with aspartate.
- Accumulation of citrulline in blood and excretion in the urine.
- Type I citrullinemia - usually becomes evident in the first few days of life.
- Type II citrullinemia - the signs and symptoms usually appear during adulthood and mainly affect the nervous system.
- Therapy – specific supplementation with arginine

Argininosuccinate lyase deficiency (argininosuccinate aciduria)

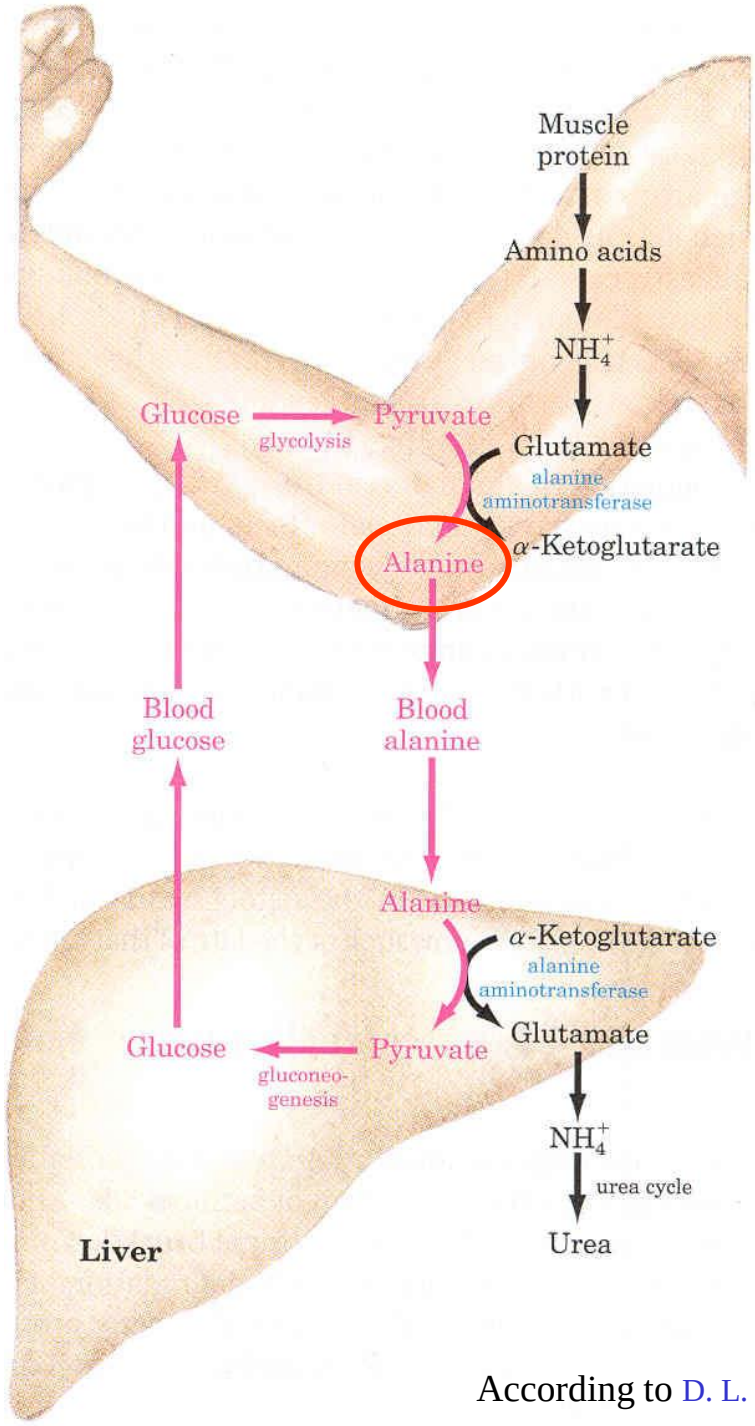
- Rare autosomal recessive disorder, argininosuccinate is excreted in large amount in urine.
- The severity of symptoms varies greatly, it is hard to evaluate the effect of therapy – useful is dietary restriction of nitrogen.

Arginase deficiency (argininemia)

- Rare autosomal recessive disorder that cause many abnormalities in development and function of CNS.
- Accumulation and excretion of arginine in urine and arginine precursors and products of arginine metabolism.
- Therapy – low nitrogen compounds diet (including essential amino acids

Which of amino acid carries the amino group from muscles to the liver?

Glucose-alanine cycle



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Ala is the carrier of ammonia and of the carbon skeleton of pyruvate from muscle to liver.
The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

According to D. L. Nelson, M. M. Cox :LEHNINGER. PRINCIPLES OF BIOCHEMISTRY Fifth edition

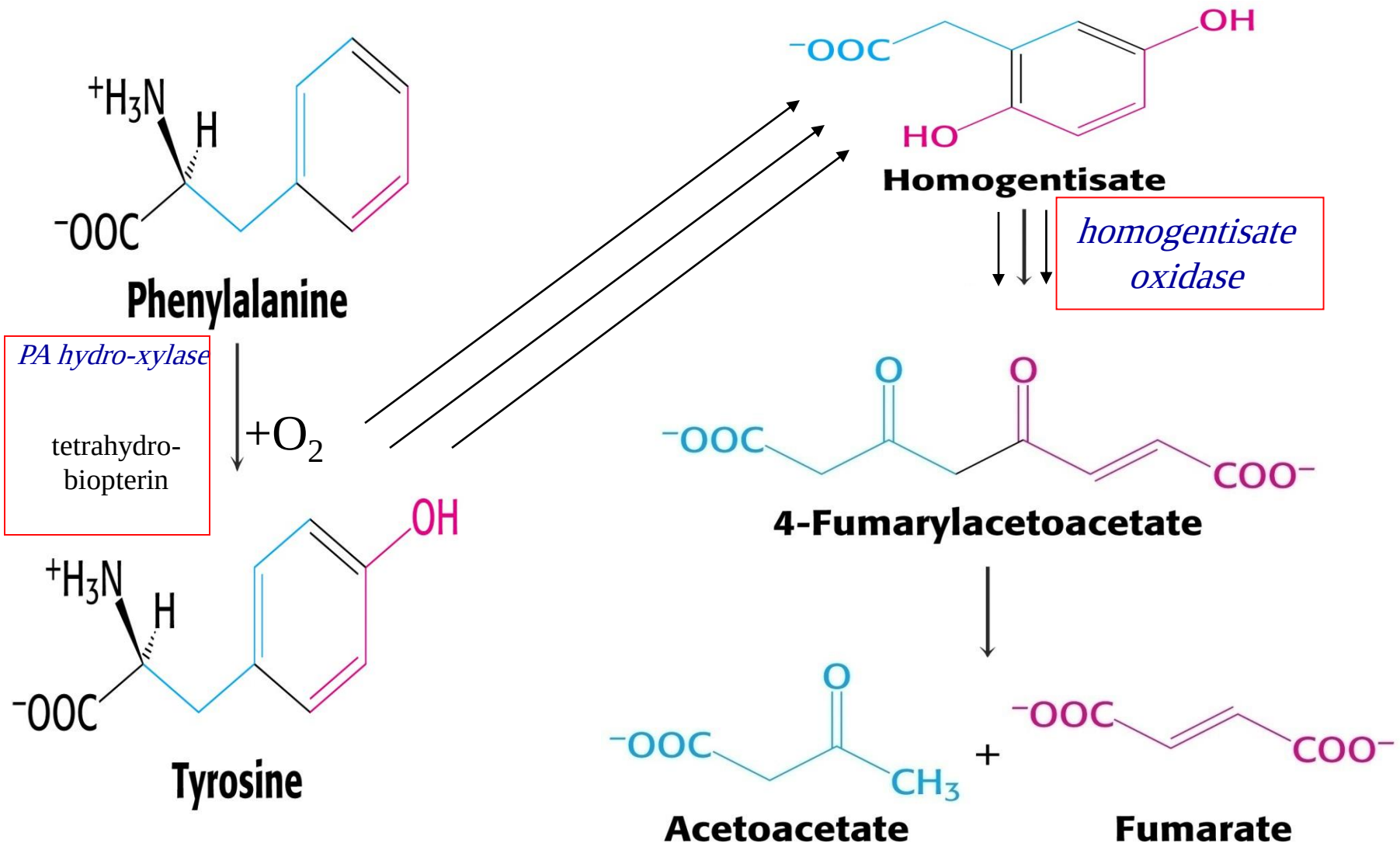
INBORN ERRORS OF AMINO ACIDS METABOLISM

Wednesday, June 29, 2016

Degradation of Aromatic Amino Acids

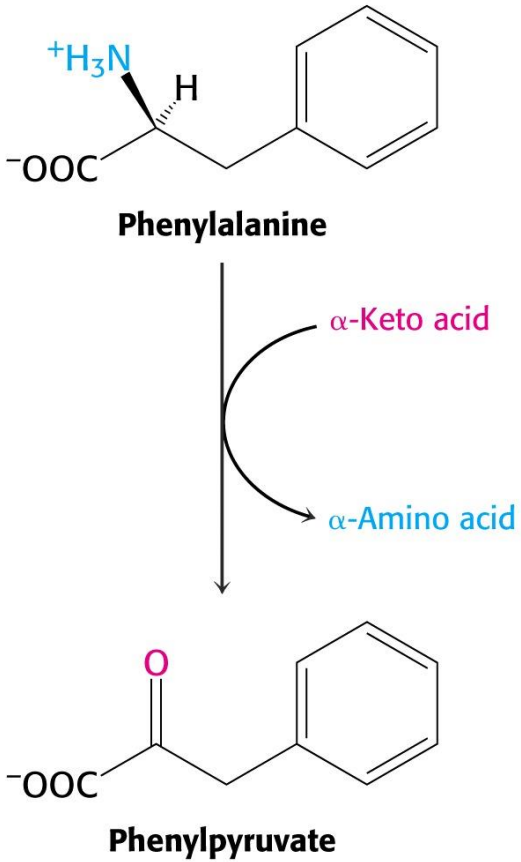
Acetoacetate, fumarate, and pyruvate — are common intermediates.

Molecular oxygen is used to break an aromatic ring.



Phenylketonuria is caused by an absence or deficiency of ***phenylalanine hydroxylase*** or of its **tetrahydrobiopterin cofactor**.

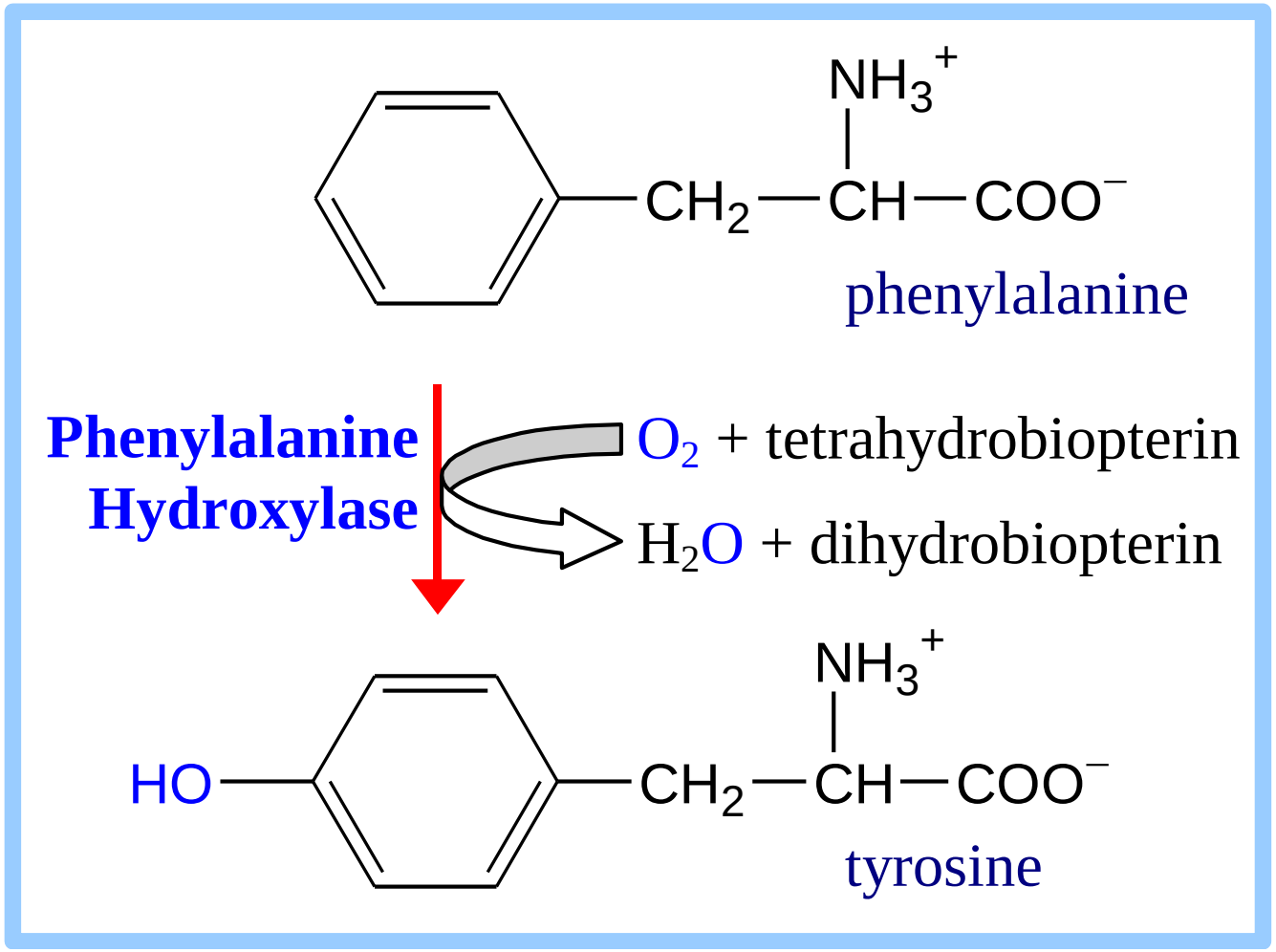
Phenylalanine accumulates in all body fluids and converts to phenylpyruvate.



- *Defect in myelination of nerves*
- *The brain weight is below normal.*
- *Mental and physical retardations.*
- *The life expectancy is drastically shortened.*

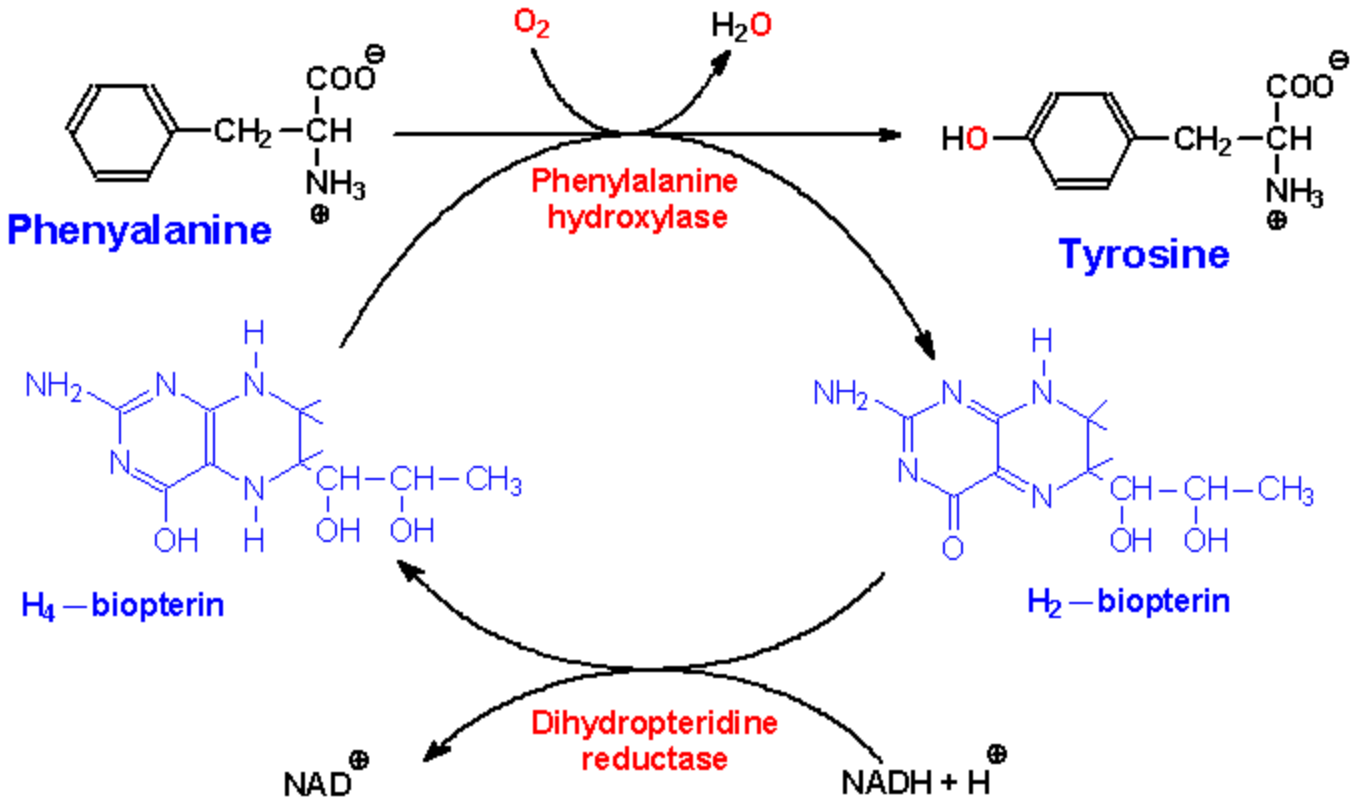
Diagnostic criteria:

- phenylalanine level in the blood
- $FeCl_3$ test
- DNA probes (prenatal)



Overall the reaction is considered a **mixed function oxidation**, because one O atom of O_2 is reduced to water while the other is incorporated into the amino acid product.

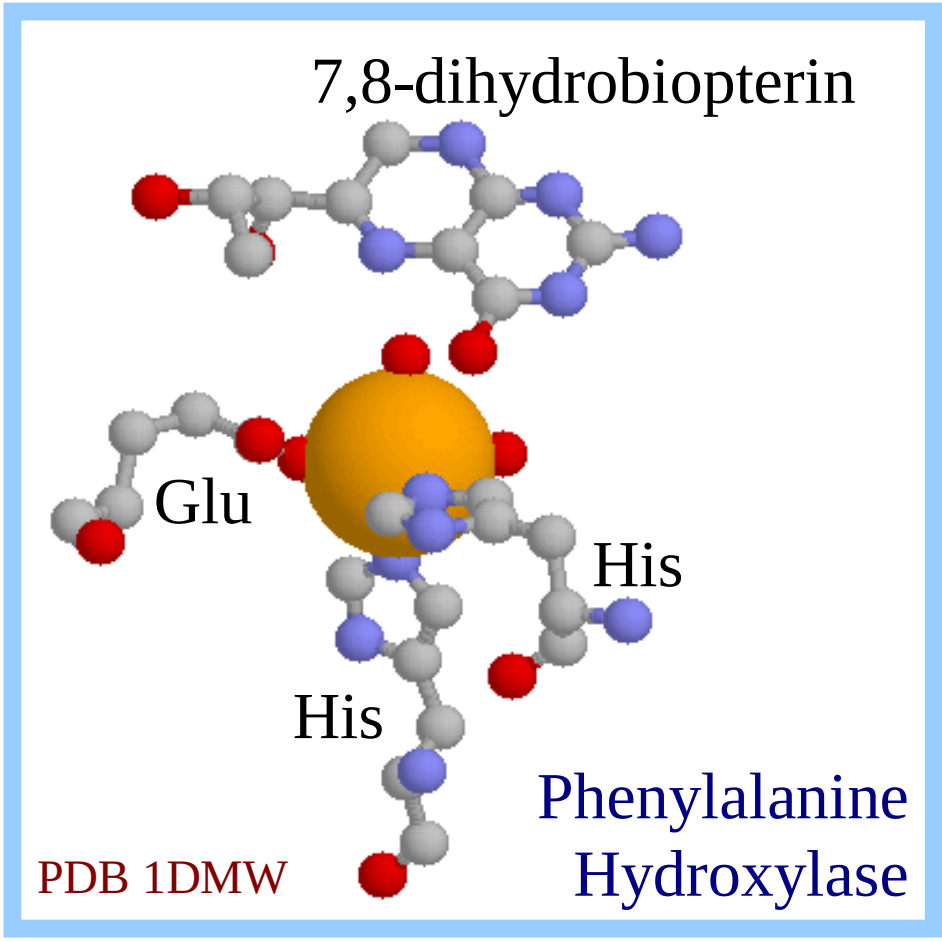
PHENYLALANINE



Phenylalanine Hydroxylase includes a non-heme **iron** atom at its active site.

X-ray crystallography has shown the following are **ligands** to the iron atom:

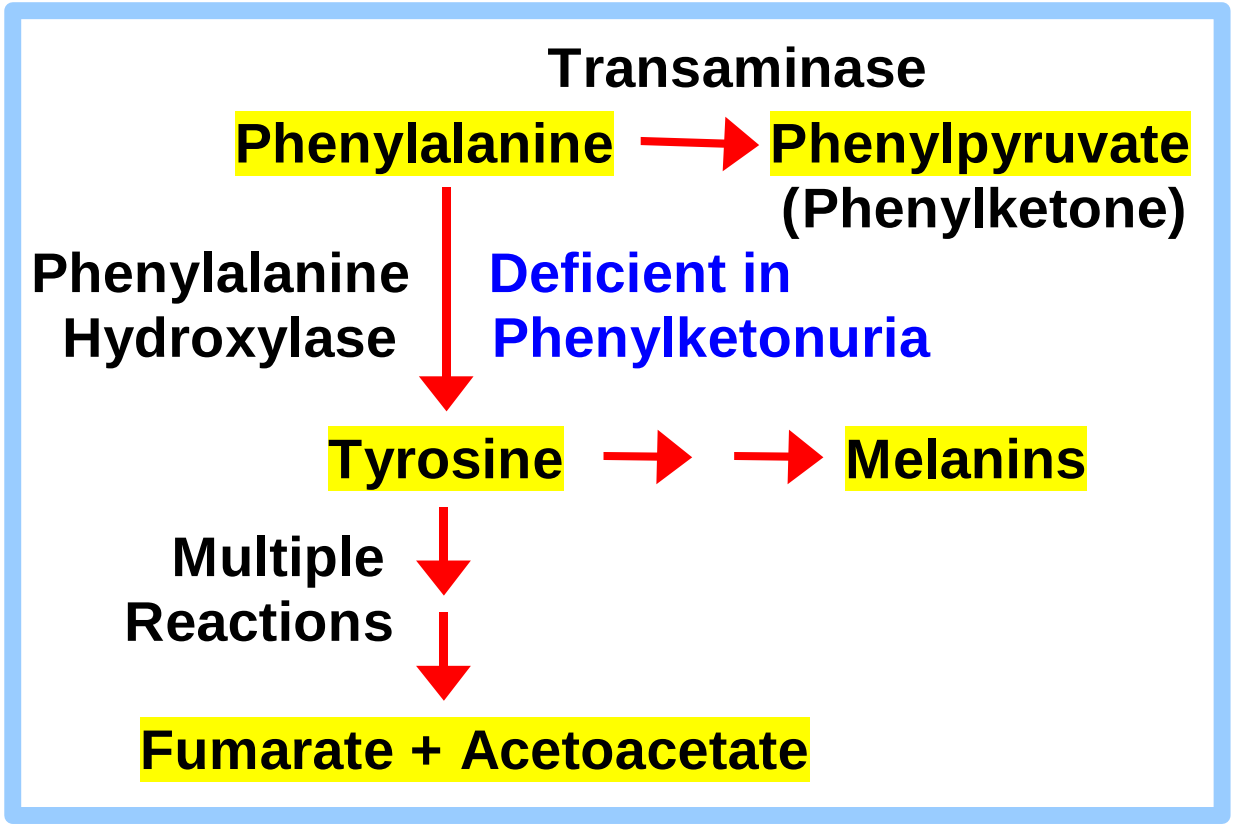
His N, Glu O & water O. (Fe shown in spacefill & ligands in ball & stick).



O_2, tetrahydrobiopterin, and the iron atom in the ferrous (Fe^{++}) oxidation state participate in the hydroxylation.

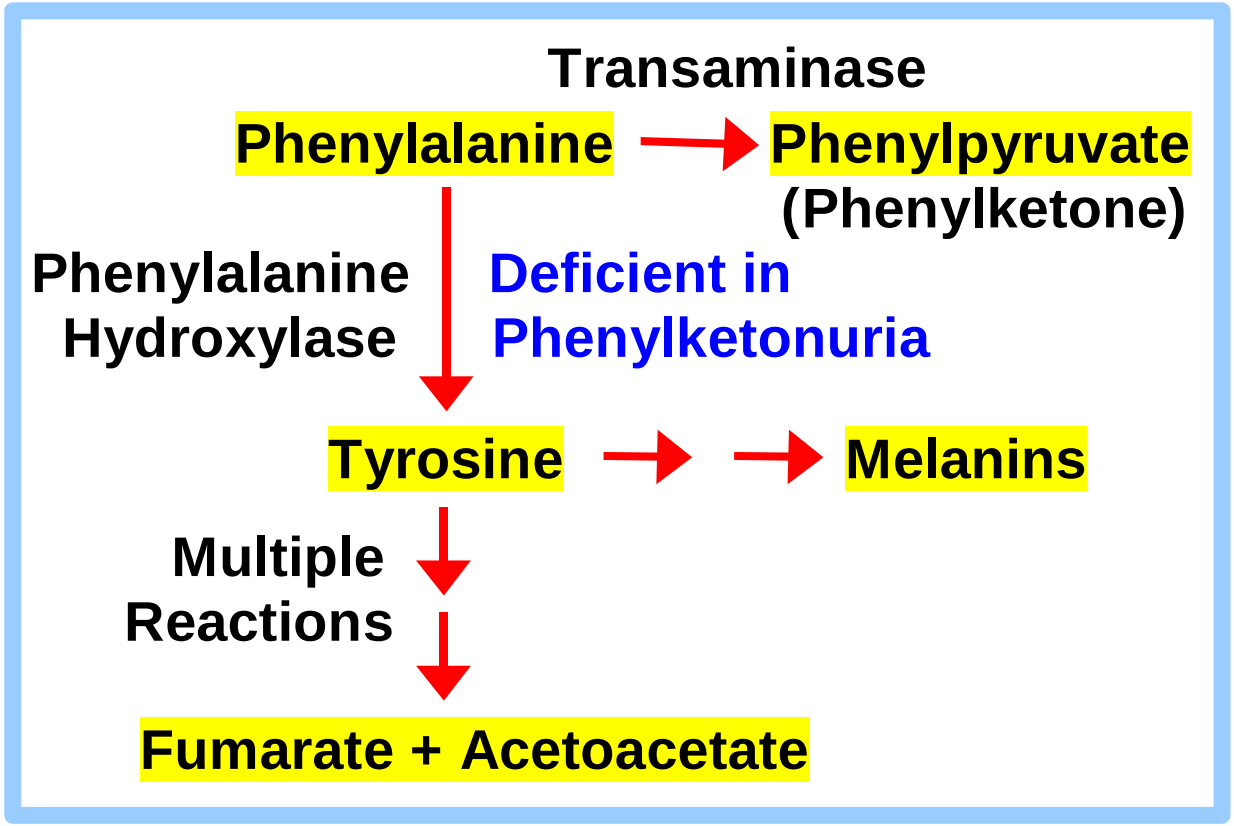
O_2 is thought to react initially with the tetrahydrobiopterin to form a peroxy intermediate.

Genetic deficiency of Phenylalanine Hydroxylase leads to the disease **phenylketonuria**.



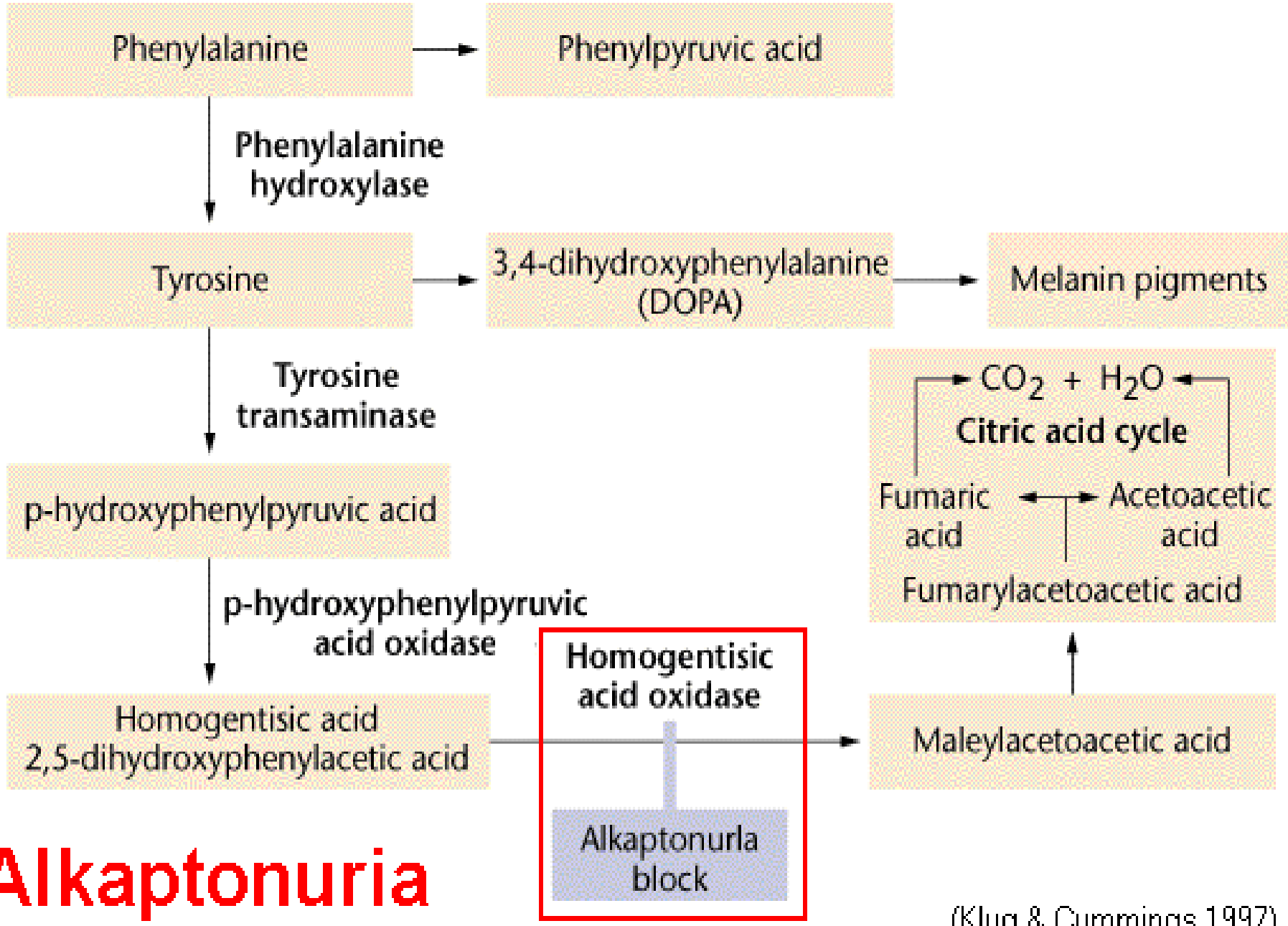
Phenylalanine & phenylpyruvate (the product of phenylalanine deamination via transaminase) accumulate in blood & urine.

Mental retardation results unless treatment begins immediately after birth. **Treatment** consists of **limiting phenylalanine intake** to levels barely adequate to support growth. **Tyrosine**, an essential nutrient for individuals with phenylketonuria, must be supplied in the diet.



Tyrosine is a precursor for synthesis of melanins and of epinephrine and norepinephrine.

High [phenylalanine] inhibits Tyrosine Hydroxylase, on the pathway for synthesis of the pigment **melanin** from tyrosine. Individuals with phenylketonuria have light skin & hair color.



Alkaptonuria

(Klug & Cummings 1997)

INBORN ERRORS OF AMINO ACIDS METABOLISM

Alcaptonuria - inherited disorder of the tyrosine metabolism caused by the absence of ***homogentisate oxidase***.

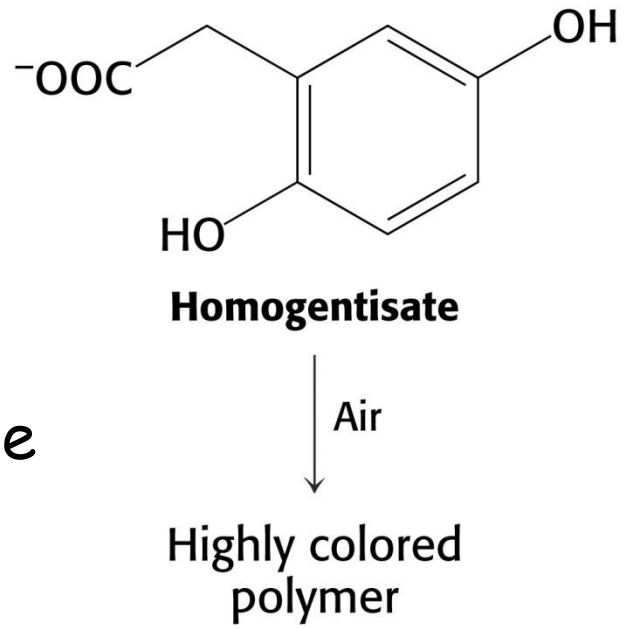
- homogentisic acid is accumulated and excreted in the urine
- turns a black color upon exposure to air

➢ **In children:**

- urine in diaper may darken

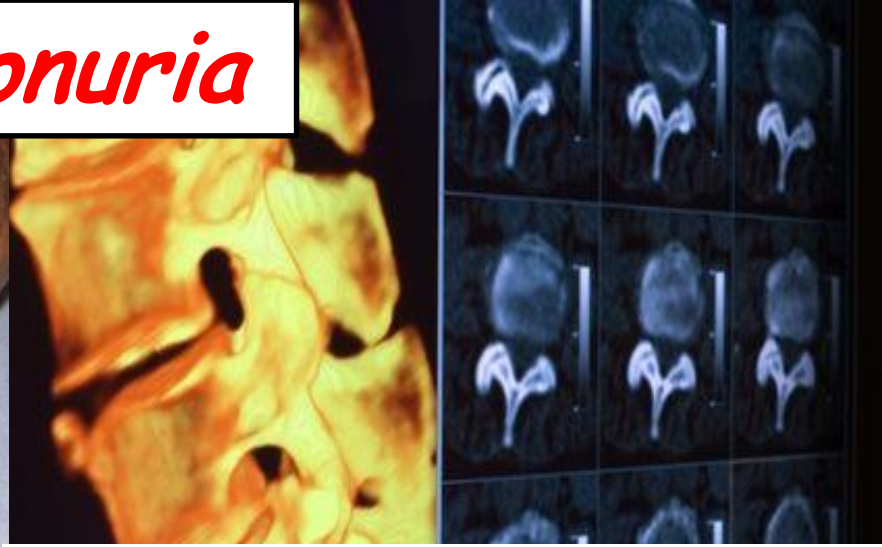
➢ **In adults:**

- darkening of the ear
- dark spots on the on the sclera and cornea
- arthritis



Alcaptonuria

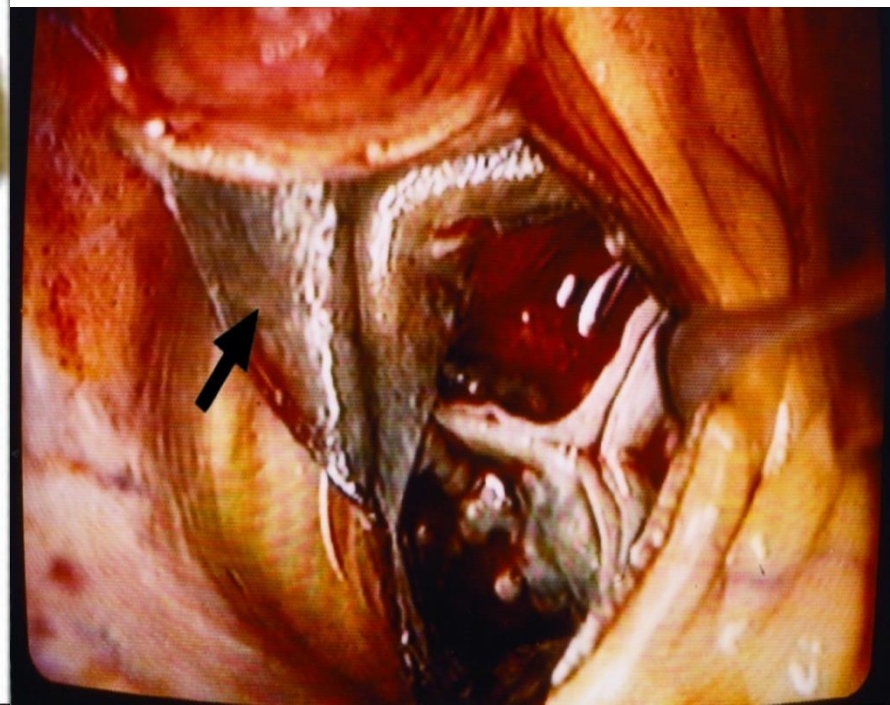
Accumulation of oxidized homogentisic acid pigment in connective tissue (ochronosis)



Arthritis of the spine is a complication of alkaptonuria ochronosis

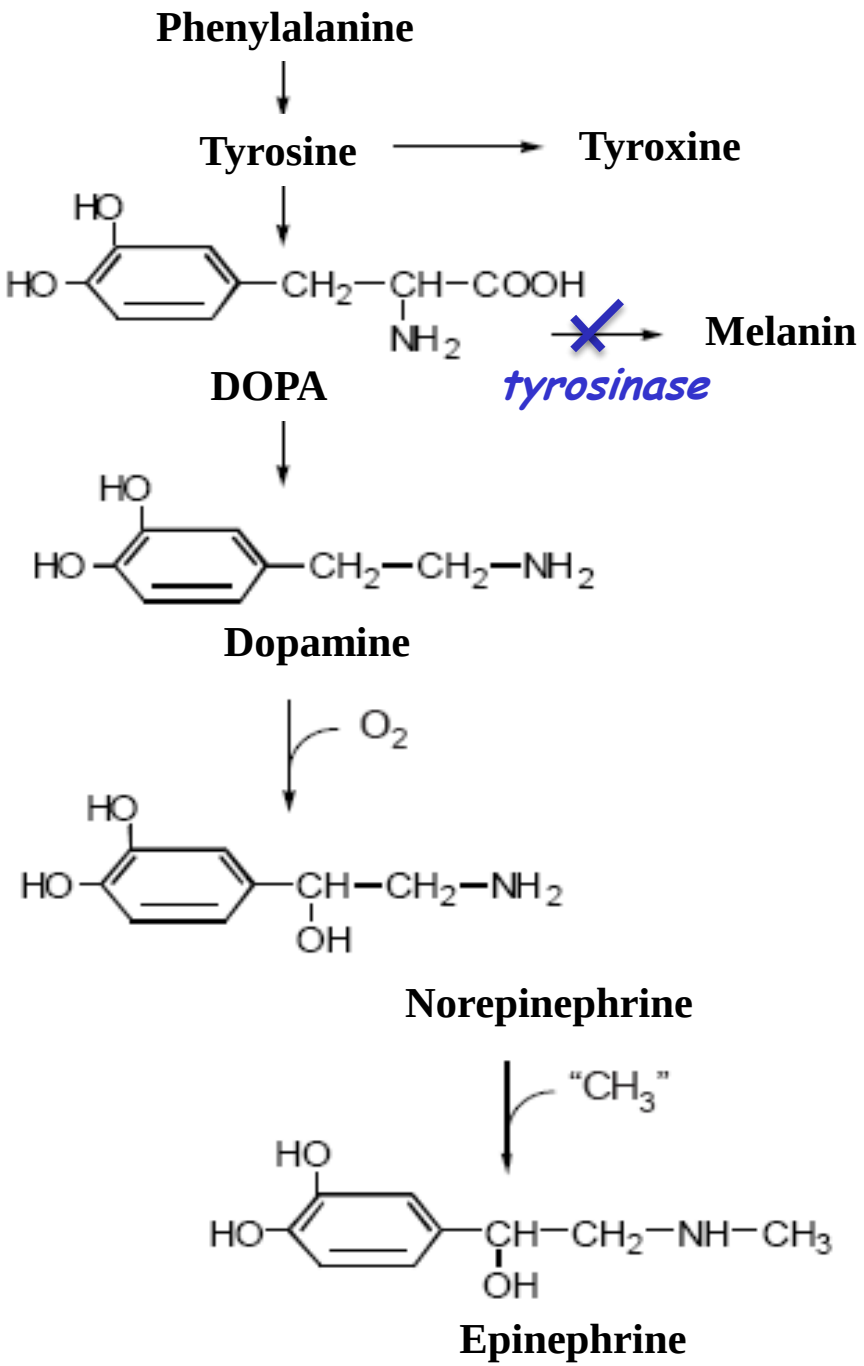
Urine turns a black color upon exposure to air

Aortic valve stenosis in alcaptonuria



Albinism – genetically determined lack or deficit of enzyme **tyrosinase**

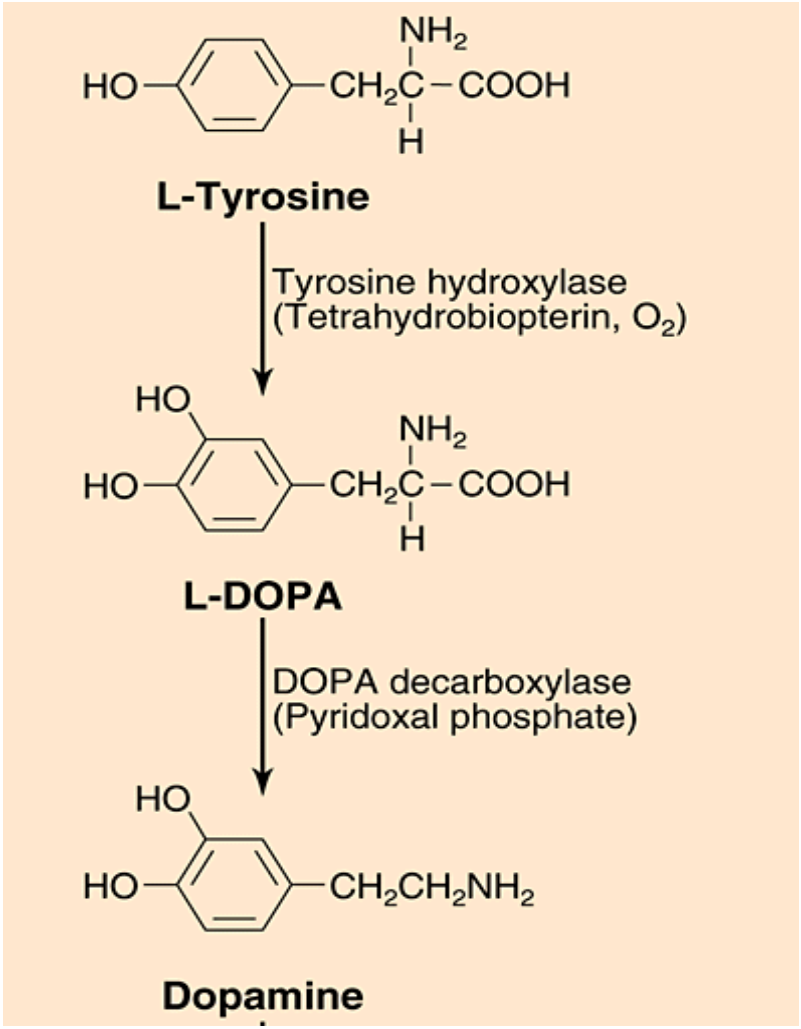
Tyrosinase in melanocytes oxidases tyrosine to DOPA and DOPA-chinone

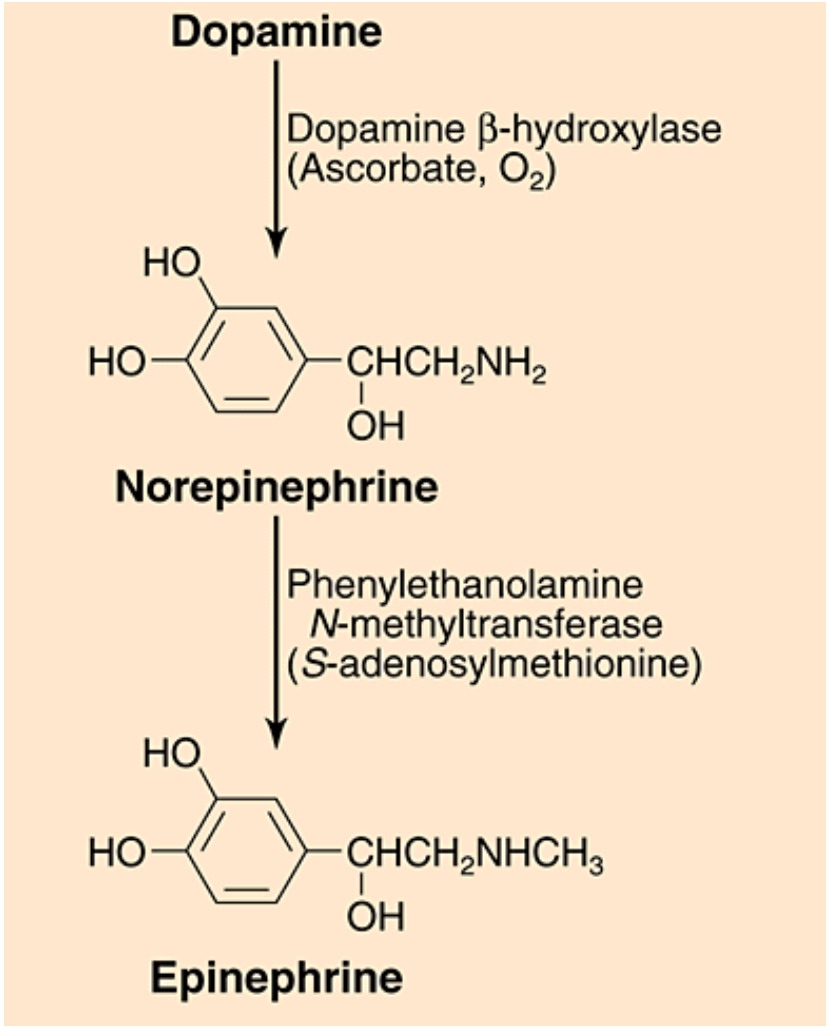


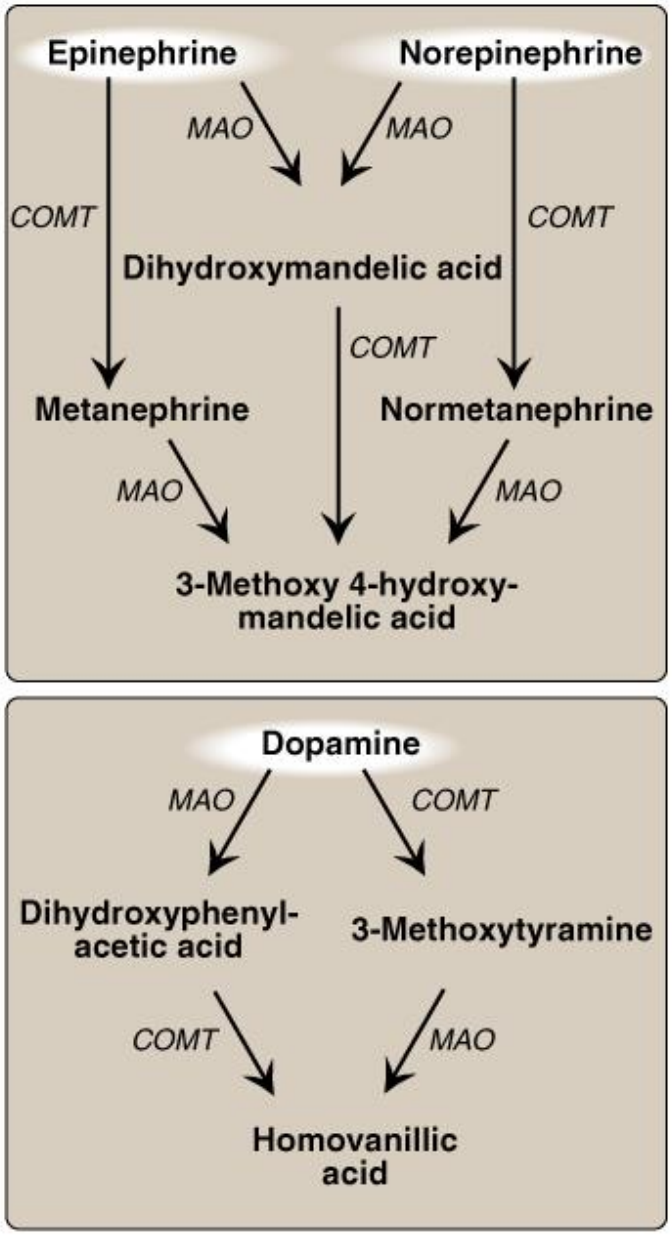
Symptoms of albinism:

- inhibition of production or lack of melanin in skin, hair, eyes
- increased sensitivity to sunlight
- increased risk of skin cancer development
- sun burns
- photophobia
- decrease of vision acuity

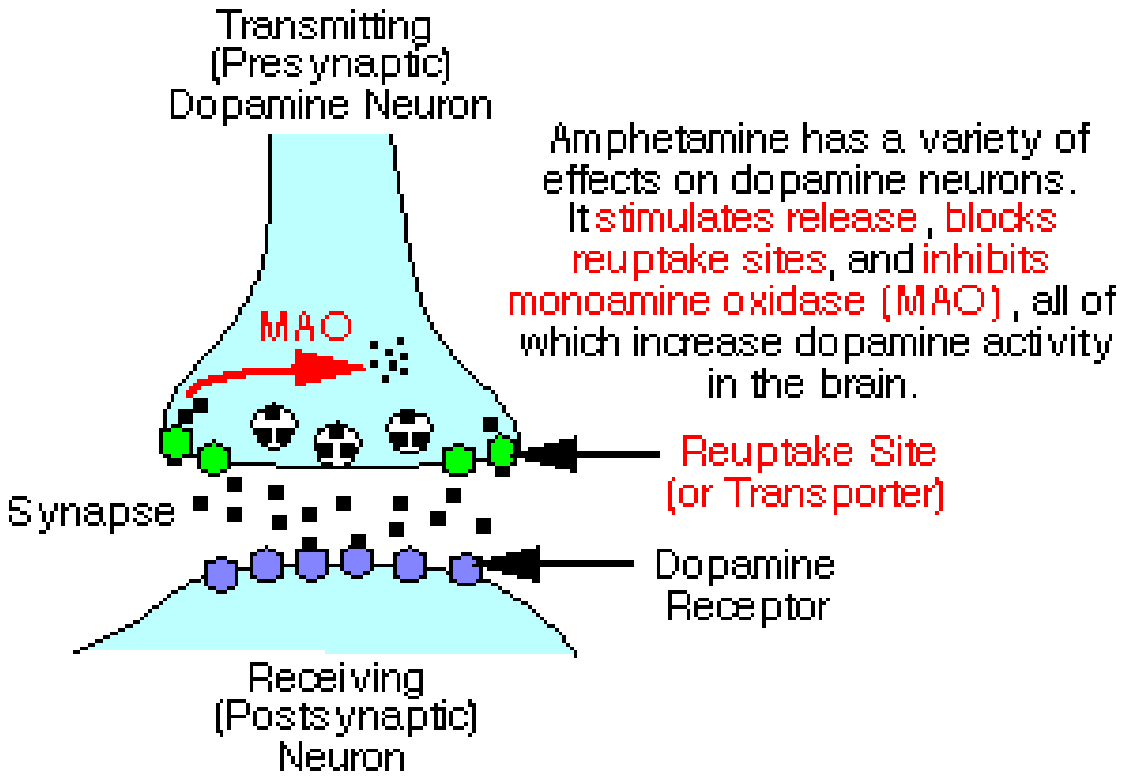
Biosynthesis of Catecholamines



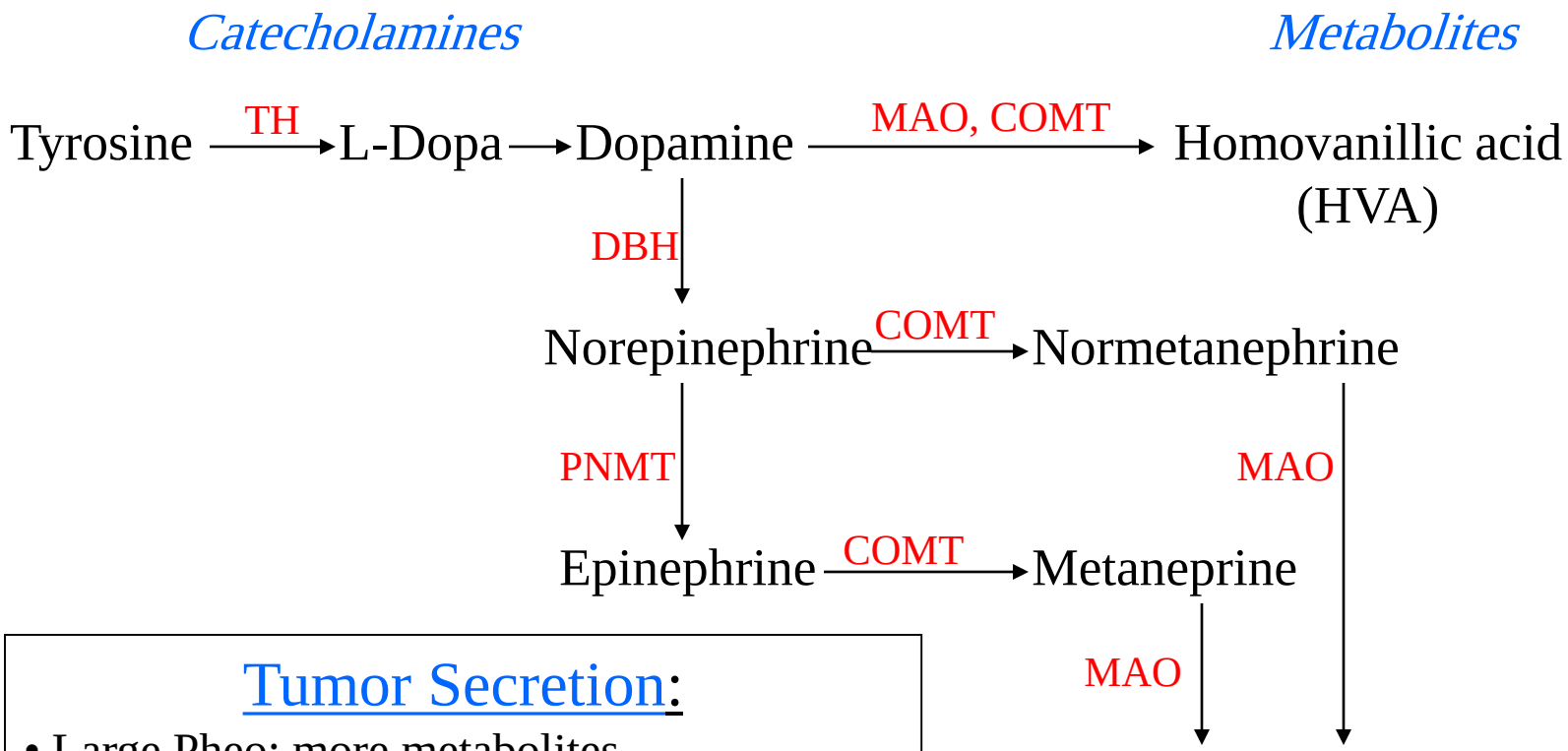



Epinephrine
Norepinephrine
MAO
MAO
COMT
COMT
Dihydroxymandelic acid
COMT
Metanephrine
Normetanephrine
MAO
MAO
3-Methoxy 4-hydroxy-
mandelic acid
Dopamine
MAO
COMT
Dihydroxyphenyl-
acetic acid
3-Methoxytyramine
COMT
MAO
Homovanillic
acid

Important fetures of catecholamine biosynthesis, uptake and signaling



1. Biosynthesis
2. Release
3. Uptake (transporter)
4. Receptor-mediated signaling
5. Catabolism



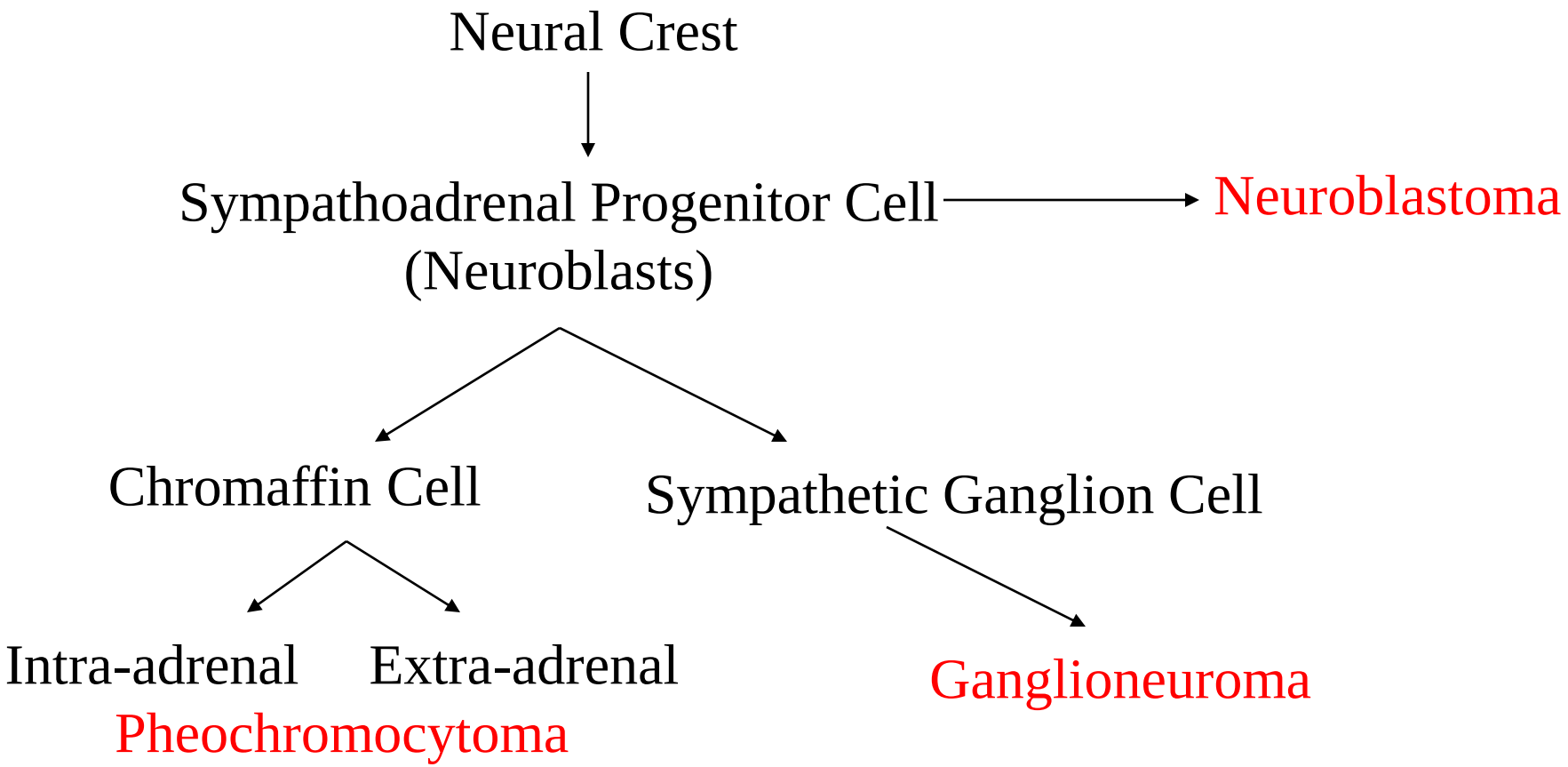
Tumor Secretion:

- Large Pheo: more metabolites
  (metabolized within tumor before release)
- Small Pheo: more catecholamines
- Sporadic Pheo: Norepi > Epi
- Familial Pheo: Epi > Norepi
- Paraganglioma: Norepi
- Cheodectoma, glomus jugulare: Norepi
- Gangioneuroma: Norepi
- Malignant Pheo: Dopamine, HVA
- Neuroblastoma: Dopamine, HVA

Adrenergic Receptors

- Alpha-Adrenergic Receptors
  - **α_1:** vasoconstriction, intestinal relaxation, uterine contraction, pupillary dilation
  - **α_2:** ↓ presynaptic NE (clonidine), platelet aggregation, vasoconstriction, ↓ insulin secretion
- Beta-Adrenergic Receptors
  - **β_1:** ↑ HR/contractility, ↑ lipolysis, ↑ renin secretion
  - **β_2:** vasodilation, bronchodilation, ↑ glycogenolysis
  - **β_3:** ↑ lipolysis, ↑ brown fat thermogenesis

Catecholamine Producing Tumors

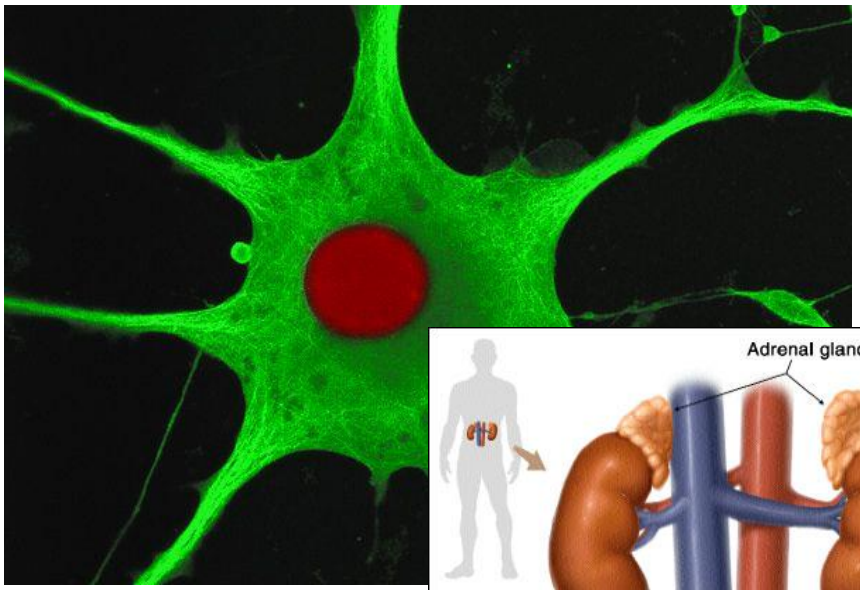


Catecholamine Producing Tumors

- Pheochromocytoma
- Paraganglioma (extra-adrenal pheo)
  - Originate in extra-adrenal sympathetic chain/chromaffin tissue
- Ganglioneuroma
  - Behave like paraganglioma biochemically
- Neuroblastoma
  - Common malignancy in children, adrenal
  - Catecholamine humoral effects usually minor
  - Rapid growth & widespread metastasis

Pheochromocytomas

- Catecholamine secreting tumor of chromaffin tissues
- Pheochromocytomas can be found anywhere chromaffin tissues exist

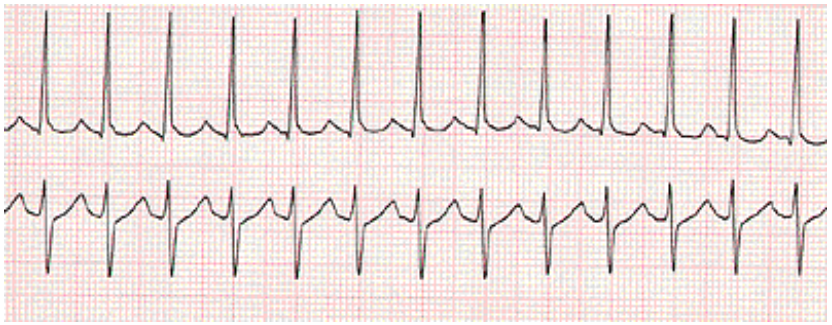


Signs and Symptoms

- HTN
- Tachycardia
- Diaphoresis
- Headaches
- Weight loss
- Palpitations
- Orthostatic hypotension

Pheochromocytoma Triad

- Diaphoresis
- Tachycardia
- Headaches
- <0.5% of hypertensive patients are diagnosed with pheochromocytoma
- Usually in patients 30-50 yrs. But can occur at any age.
- Male=Female



Diagnosis

- Blood catecholamine levels
- Pheochromocytomas are found on CT and MRI.
- **24hr urine collection**
- free norepinephrine
- catecholamines have a short half life.
- False positives: same as for 24h urine testing, also with diuretics, smoking
- Plasma total catechols > 11.8 nM (2000 pg/mL)
- SEN 85% SPEC 80%
- Drawn with patient fasting, supine, with an indwelling catheter in place > 30 min
-

Diagnosis

- **Vanillymandelic acid (VMA).**
- Final common product of both catecholamine metabolic pathways
- Often used as an initial test specificity and sensitivity. ↓ cost

Plasma Metanephrines

- Not postural dependent: can draw normally
- Secreted continuously by pheo
- SEN 99% SPEC 89%
- False Positive: acetaminophen

Biochemical Tests: Summary

	SEN	SPEC
$U_{catechols}$	83%	88%
$U_{total\ metanephrines}$	76%	94%
$U_{catechols+metaneph}$	90%	98%
U_{VMA}	63%	94%
Plasma catecholamines	85%	80%
Plasma metanephrines	99%	89%

Localization: Imaging

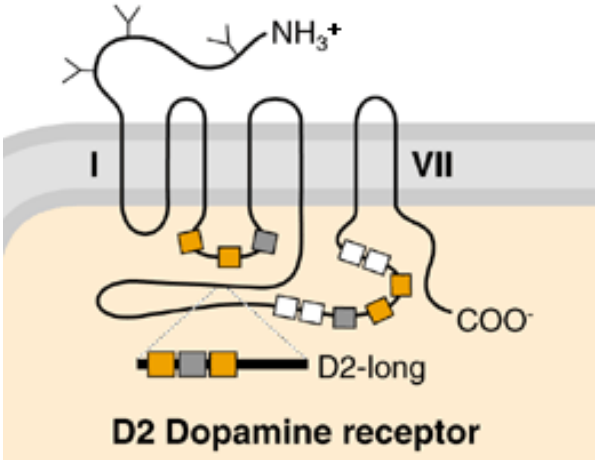
- CT abdomen
  - Adrenal pheo SEN 93-100%
  - Extra-adrenal pheo SEN 90%
- MRI
  - > SEN than CT for extra-adrenal pheo

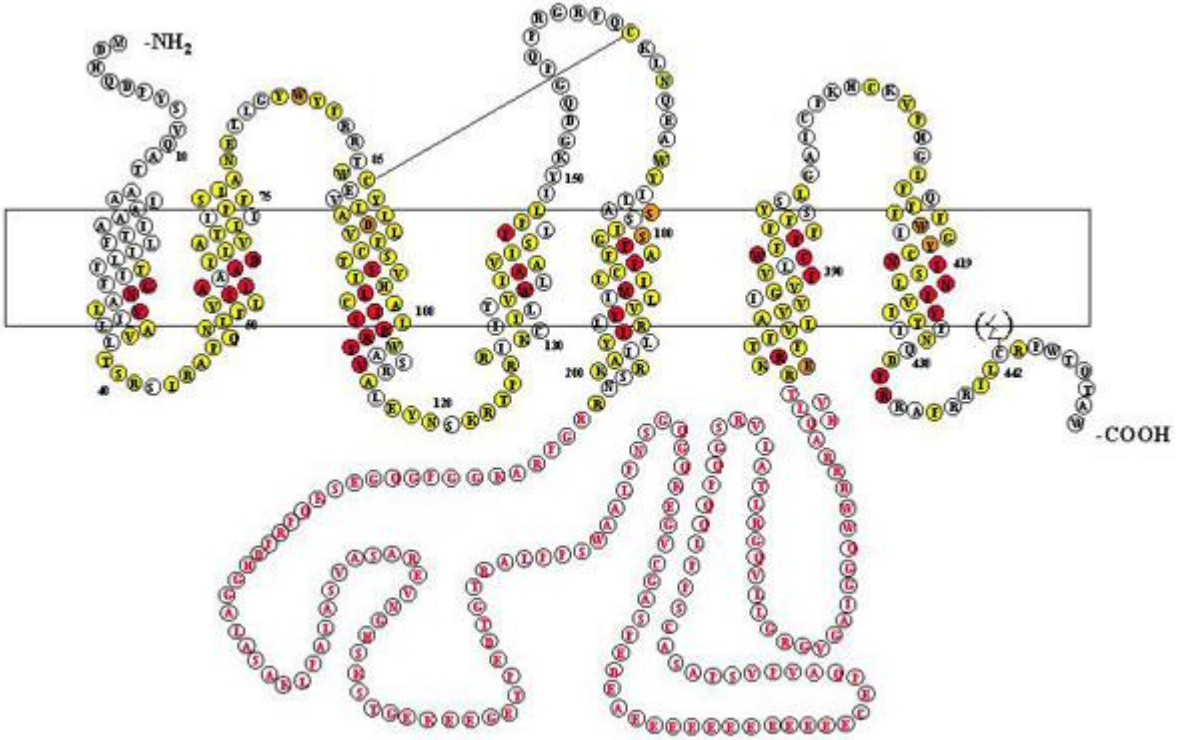
Classification of Dopamine receptors

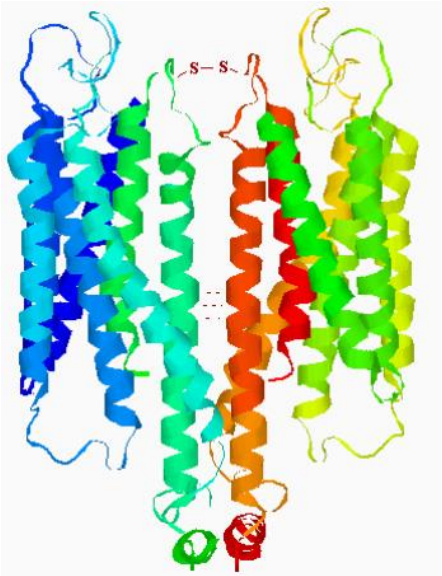
TABLE 12-3. PROPERTIES OF CLONED DOPAMINE RECEPTOR SUBTYPES

	D1	D5	D25/D2L	D3	D4
Amino acids (human)	446	477	415/444	400	387
Chromosome	5	4	11	3	11
Effector pathways	↑cAMP	↑cAMP	↓cAMP ↑K^+ channel ↓Ca^{2+} channel	↓cAMP	↓cAMP ↑K^+ channel
mRNA distribution	Caudate putamen, nucleus accumbens, olfactory tubercle	Hippocampus, hypothalamus	Caudate putamen, nucleus accumbens, olfactory tubercle	Olfactory tubercle, hypothalamus, nucleus accumbens	Frontal cortex, medulla, midbrain

structure of dopamine D_2 receptor





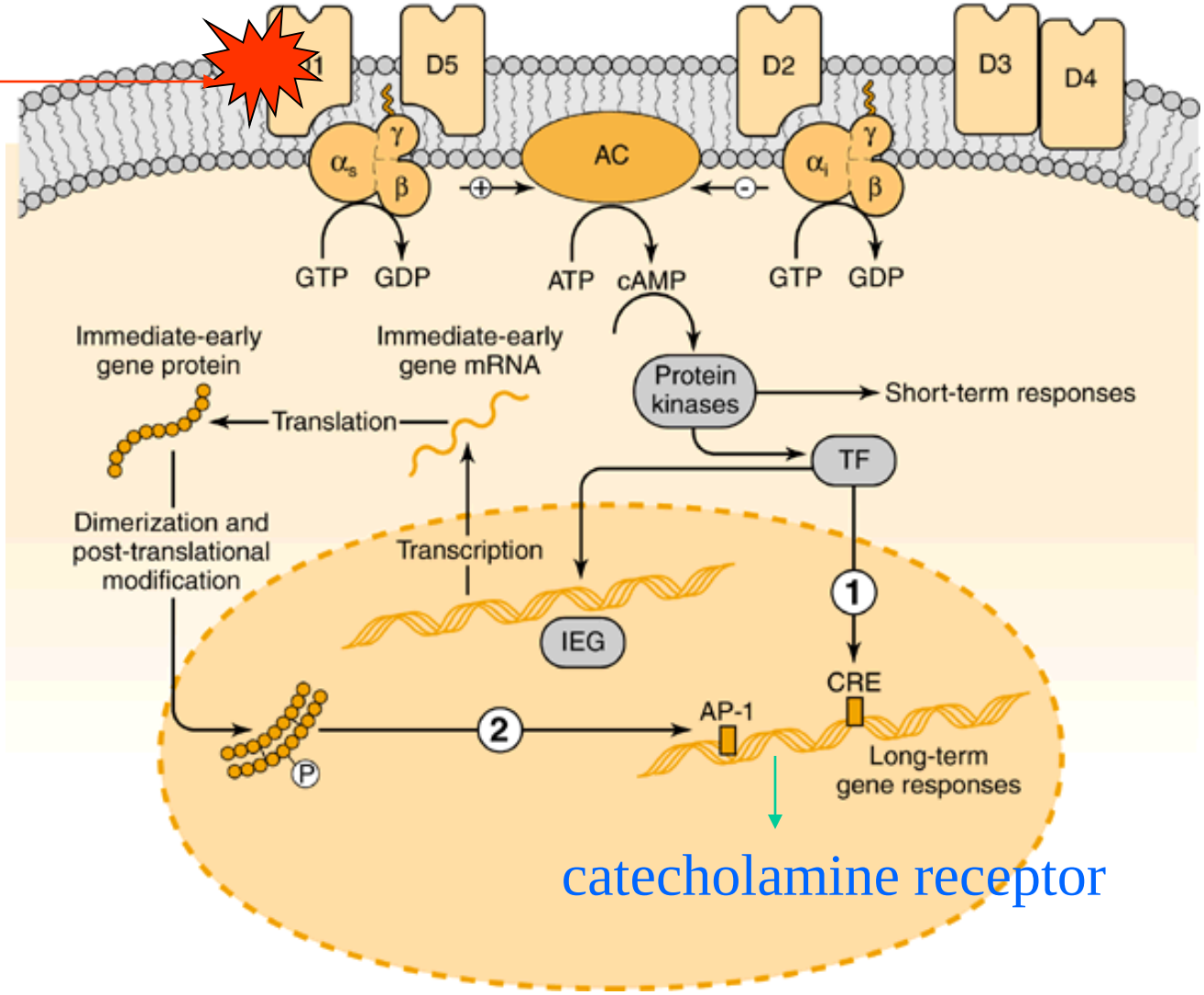


Dynamics of catecholamine receptors

(up-regulation and down-regulation)

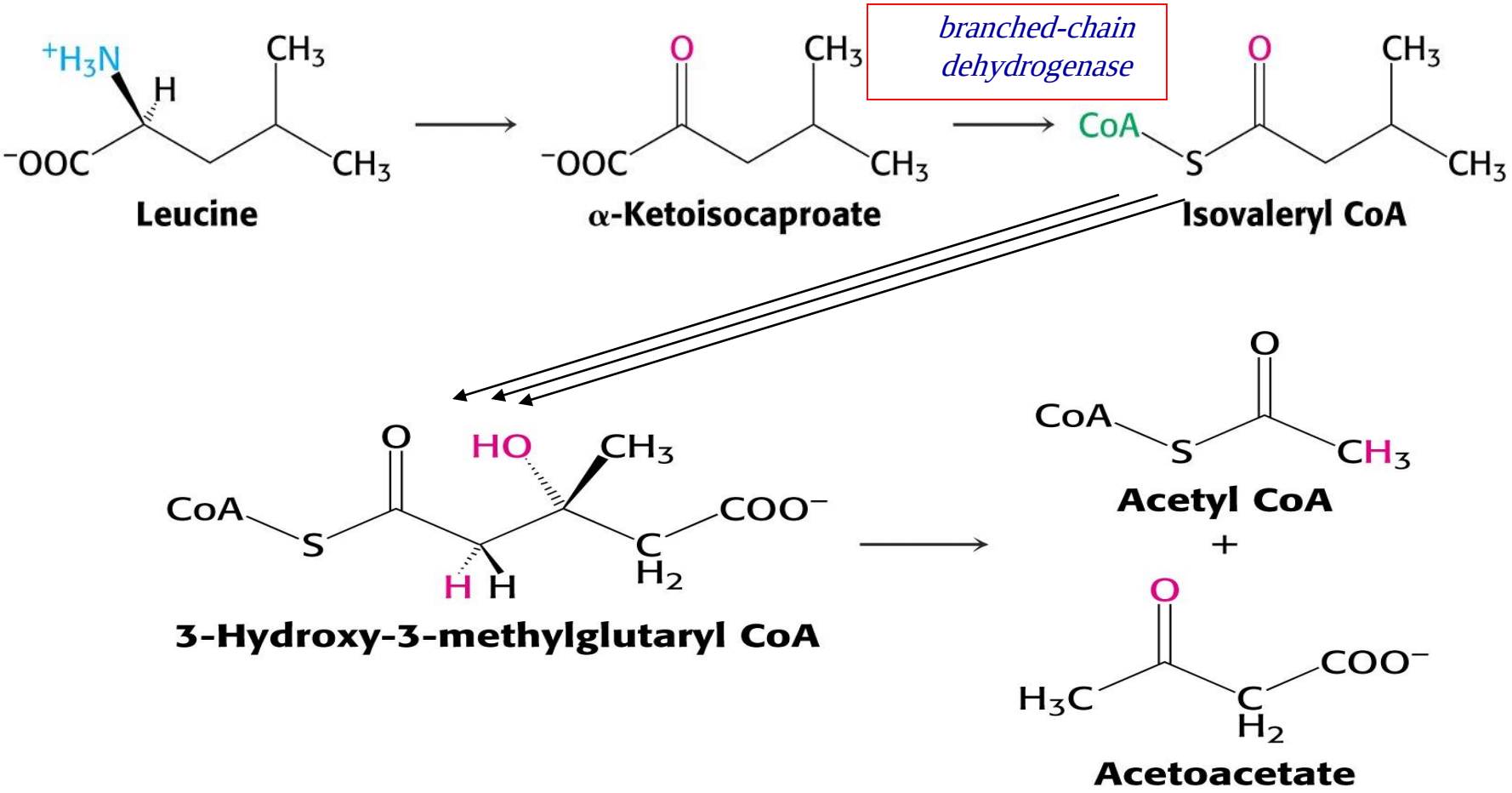
agonist

antagonist



catecholamine receptor

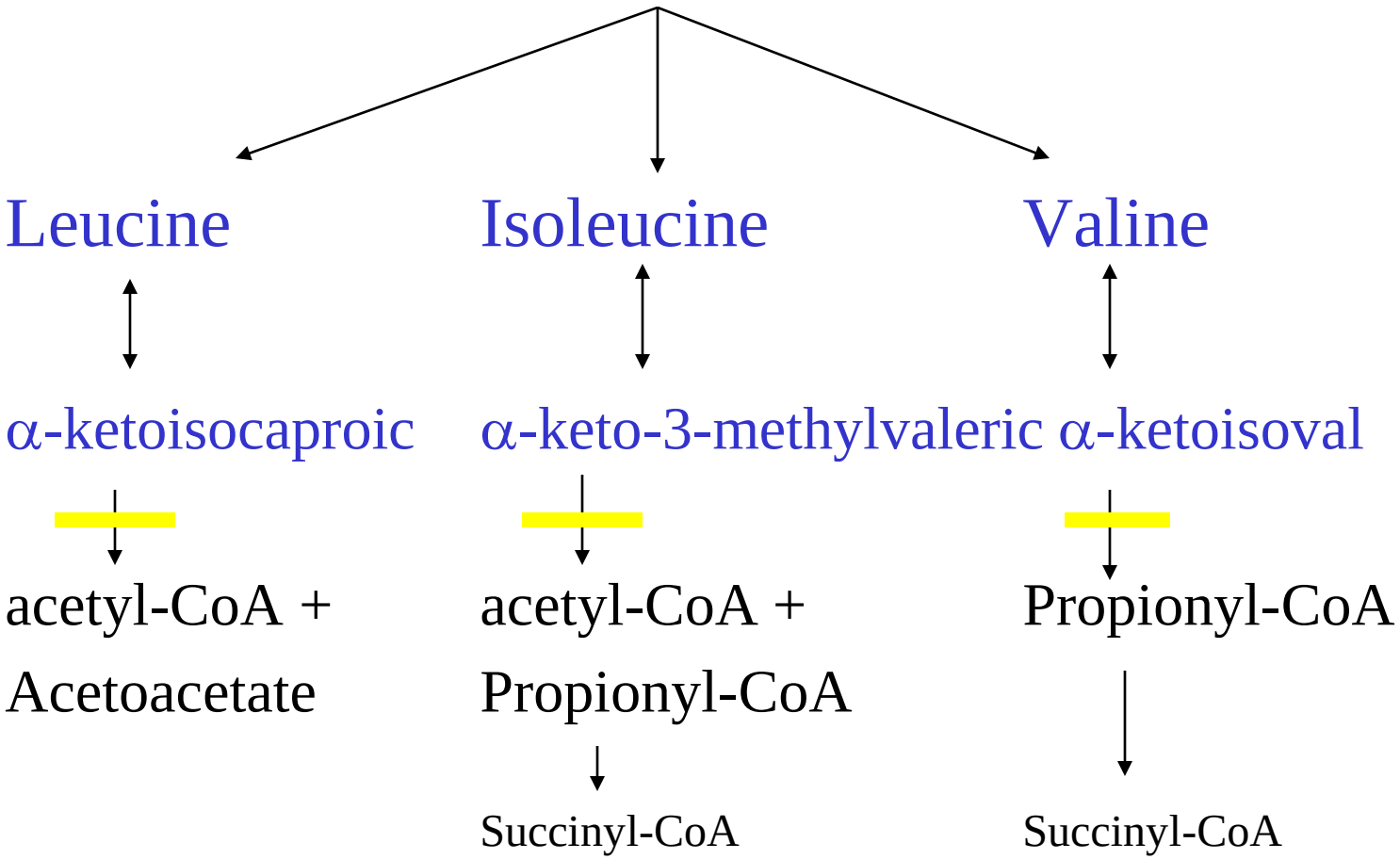
The Conversion of Branched-Chain Amino Acids



The degradative pathways of valine and isoleucine resemble that of leucine.

Isoleucine yields acetyl CoA and propionyl CoA

Valine yields CO_2 and propionyl CoA.


Dietary Protein
Catabolized Tissue Protein
Leucine
Isoleucine
Valine
α-ketoisocaproic
α-keto-3-methylvaleric
α-ketoisoval
acetyl-CoA +
Acetoacetate
acetyl-CoA +
Propionyl-CoA
Propionyl-CoA
Succinyl-CoA
Succinyl-CoA
branched-chain α-ketoacid dehydrogenase complex

Maple syrup urine disease **-** the disorder of the oxidative decarboxylation of α-ketoacids derived from valine, isoleucine, and leucine caused by the missing or defect of ***branched-chain dehydrogenase***.

The levels of branched-chain amino acids and corresponding α-ketoacids are markedly elevated in both blood and urine.

The urine has the odor of maple syrup

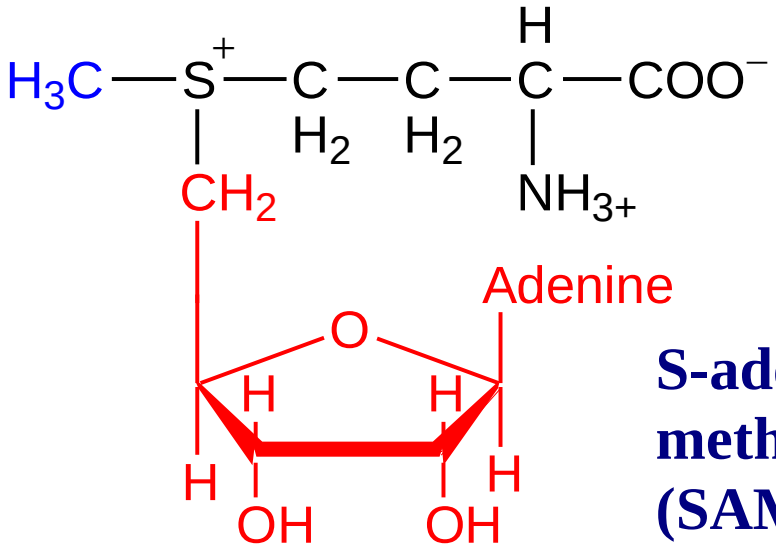
The early symptoms:

- lethargy
- ketoacidosis
- unrecognized disease leads to seizures, coma, and death
- mental and physical retardation

SAM is a **methyl group donor** in synthetic reactions.

The resulting ***S*-adenosylhomocysteine** is hydrolyzed to **homocysteine**.

Homocysteine may be catabolized via a complex pathway to **cysteine** & **succinyl-CoA**.

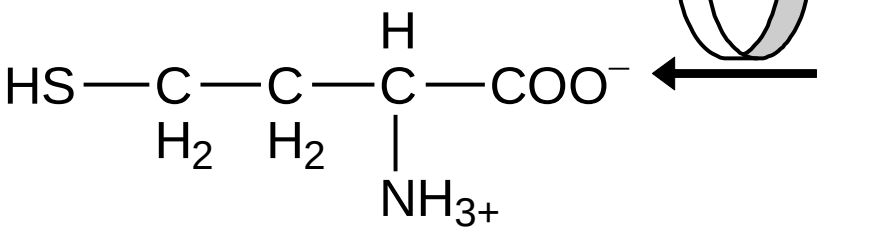


S-adenosyl-methionine (SAM)

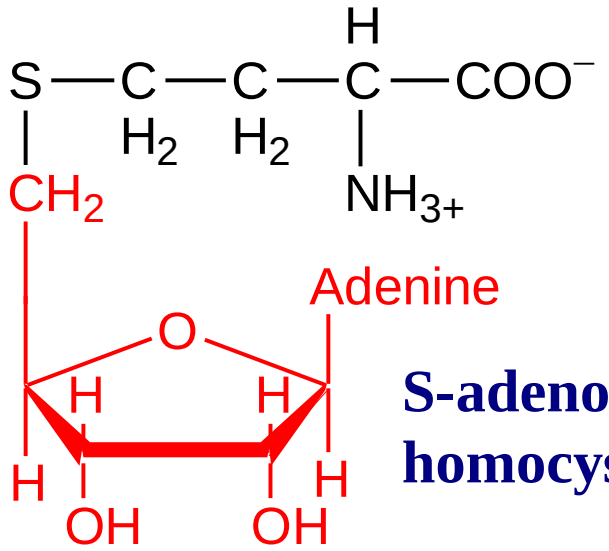
acceptor

methylated acceptor

adenosine H_2O



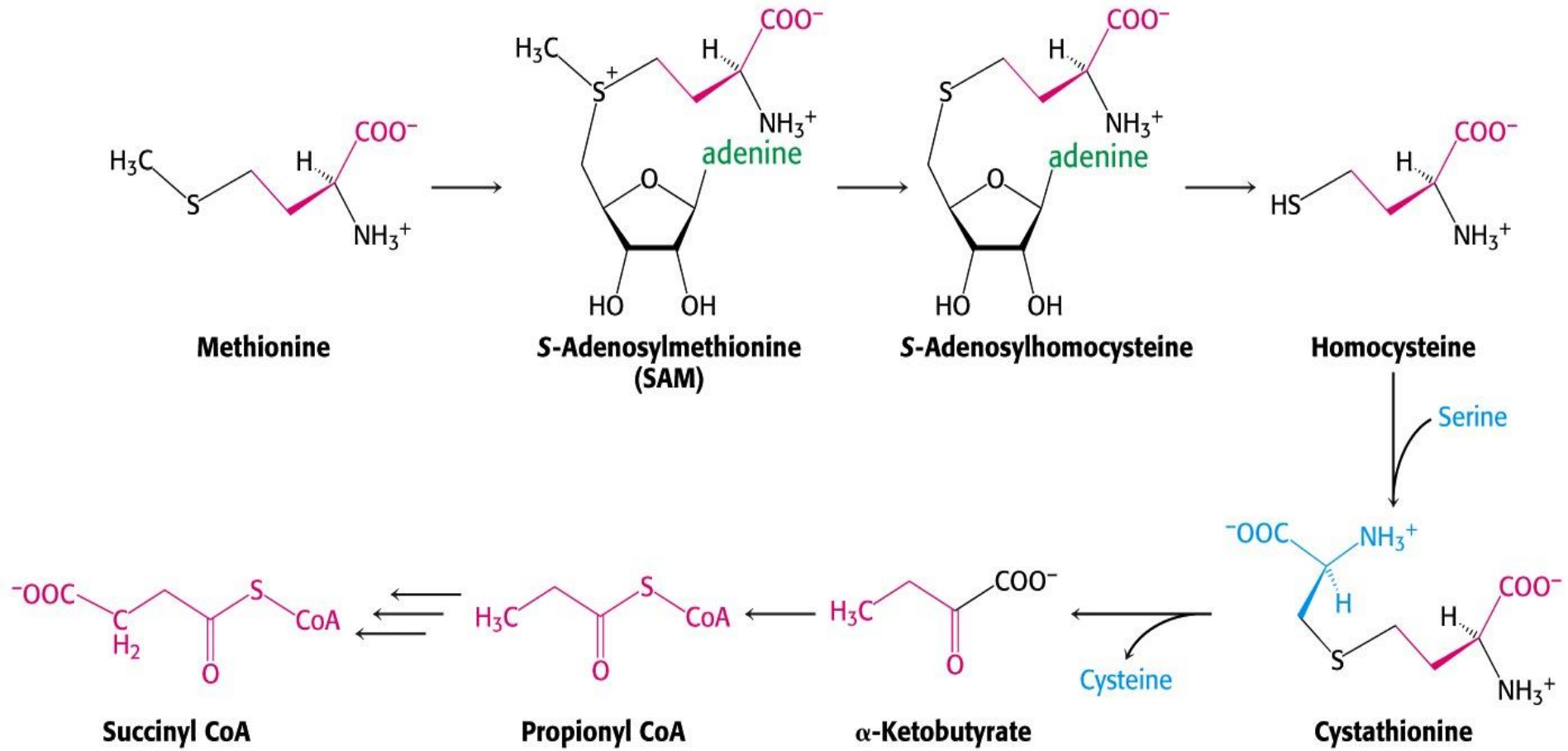
homocysteine



S-adenosyl-homocysteine

Methionine Degradation

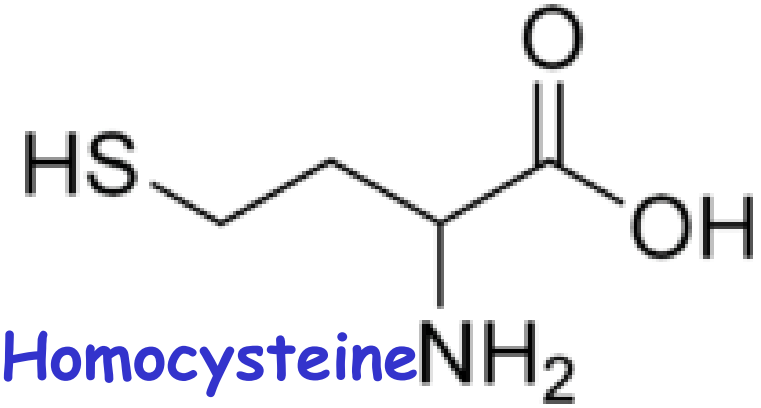
S-adenosylmethionine (SAM) - a common methyl donor in the cell



Homocysteine (< 15 µmol/L)

Hyperhomocysteinemia can results in:

- Vascular diseases, endothelial dysfunction, atherosclerosis, thrombophilia
- Skeletal anomalies
- retardation of mental development
- Alzheimer's disease
- Kidneys insufficiency
- Colorectal cancer



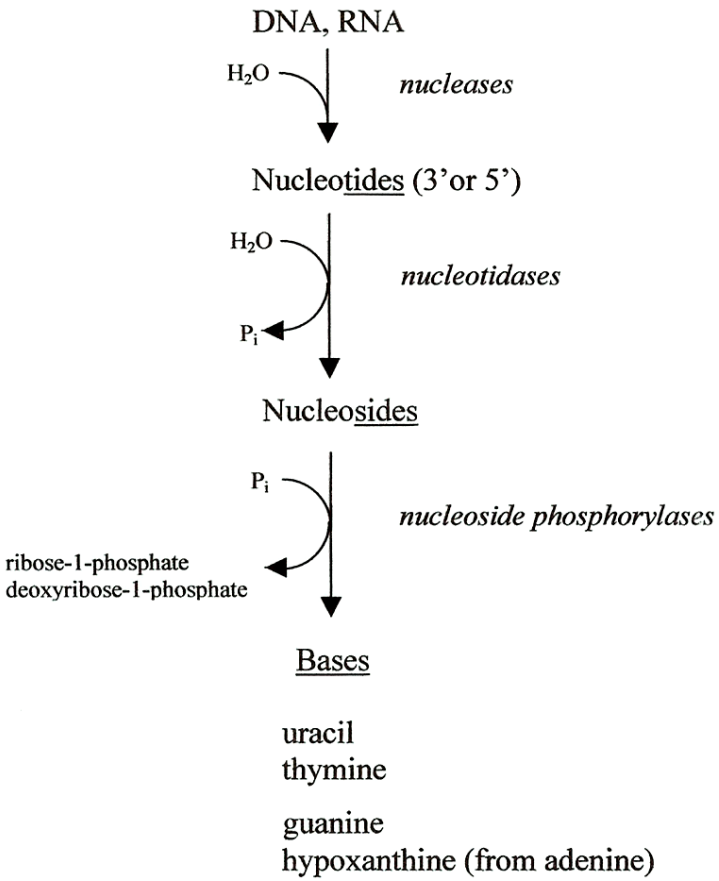
Homocysteine

HOMOCYSTINURIA AND HEART DISEASE

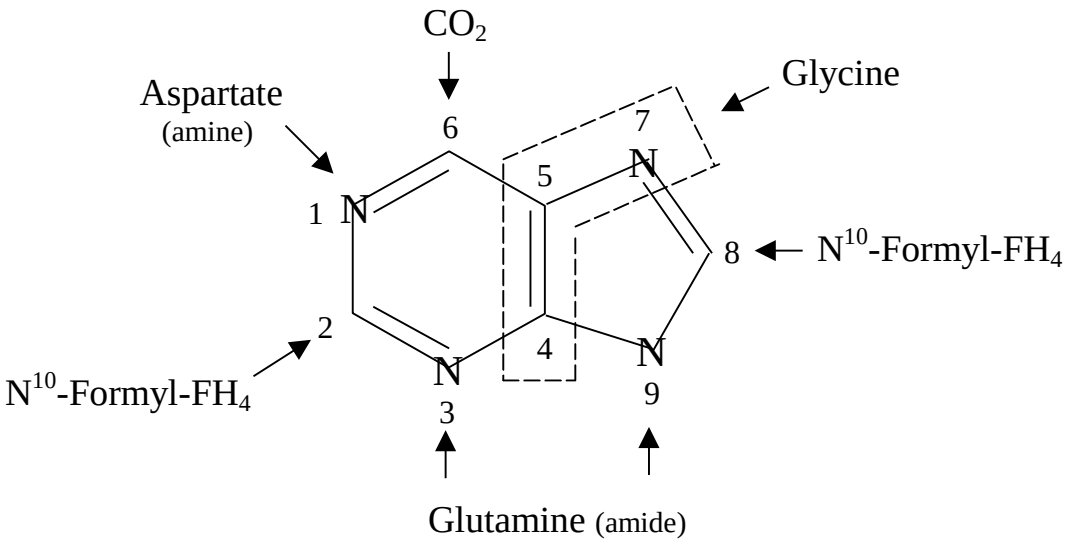
- Homocystinuria: homocystinmethyl transferase deficiency
- some patients with arteriosclerosis have elevated homocysteine
- low plasma B vitamins and low dietary intake show increased risk for heart disease
- folate/B_{12} deficiency common in high risk populations (elderly, smokers)
- B vitamins decrease plasma homocysteine , homocysteine may oxidatively damage lipoproteins and endothelia of vessel walls
- Cystinuria : cystin-lysinuria , membrane system transport deficiency
- Cystinosis : lysosomal function deficiency
- Homocystinuria :cystathionine synthase deficiency
- Cystathioninuria : cystathionine lyase defieciency + B6 deficiency

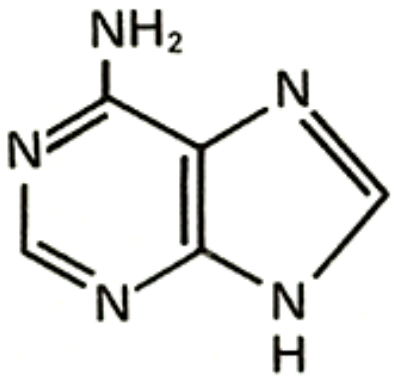
Purine and Pyrimidine Metabolism

DNA and RNA Degradation



Source of each atom in the purine ring



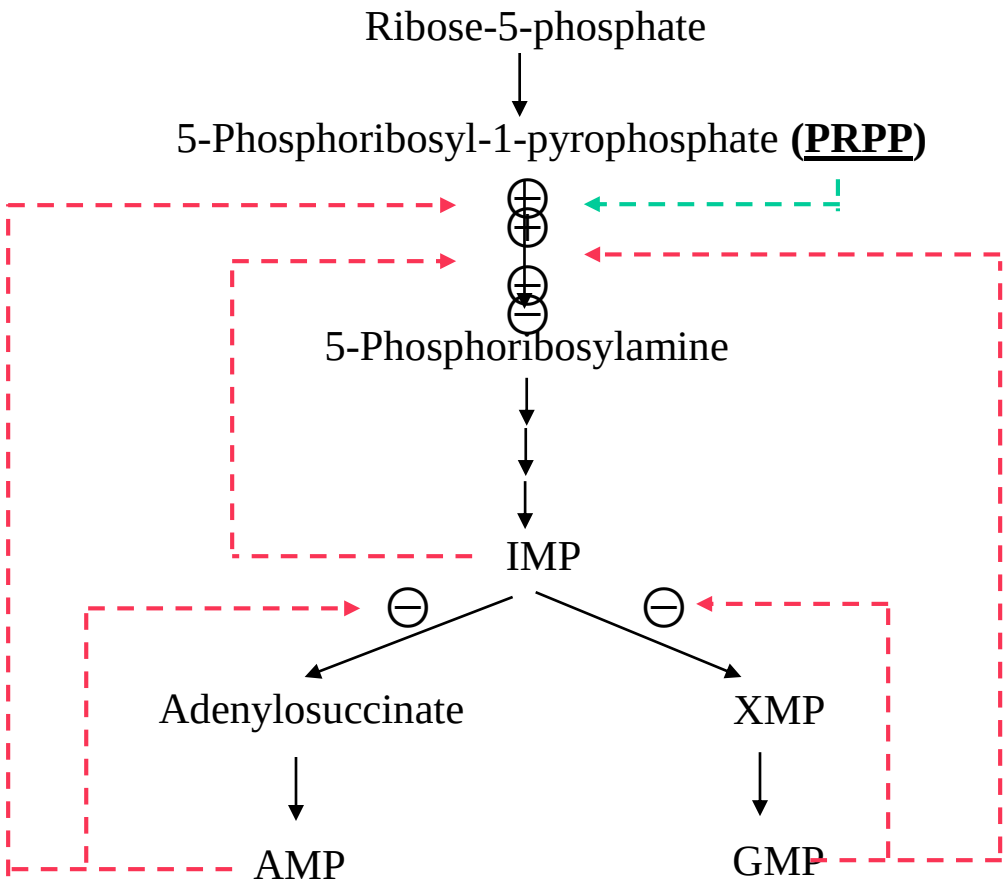


Adenine (A)

Guanine (G)

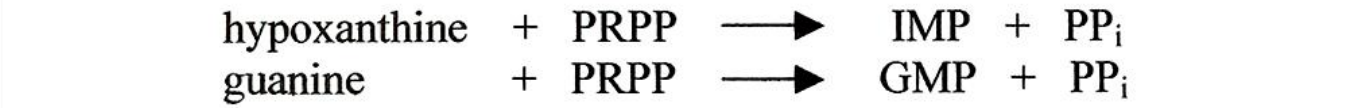
Major Bases

Summary and Regulation



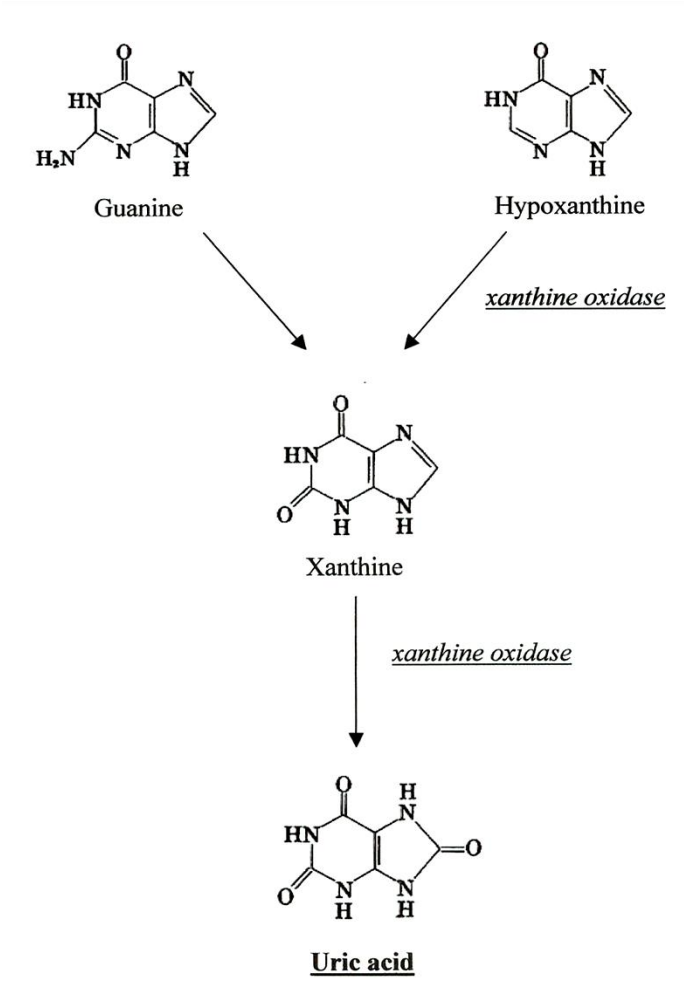
"Salvage Pathway" for Purines
(~90%)

Enzyme: Hypoxanthine-guanine phosphoribosyltransferase (HGPRTase)



Lesch-Nyhan Syndrome

Degradation of Purines



URATE, HYPERURICEMIA & GOUT

- Urate: end product of purine metabolism

- Hyperuricemia: serum urate > urate solubility (> 6.8 mg/dl)

- Gout: deposition of monosodium urate crystals in tissues
- Hyperuricemia caused by

  Overproduction

  Underexcretion

GOUT: A Chronic Disease of 4 stages

- Asymptomatic hyperuricemia
- Acute Flares of crystallization
- Intervals between flares
- Advanced Gout & Complications

ACUTE GOUTY FLARES

- Abrupt onset of severe joint inflammation, often nocturnal;

  Warmth, swelling, erythema, & pain;

  Possibly fever
- 90% 1st attacks are monoarticular

SITES OF ACUTE FLARES

- 90% of gout patients eventually have podagra : 1st MTP joint

TOPHI

- Solid urate deposits in tissues

TOPHI RISK FACTORS

- Long duration of hyperuricemia
- Higher serum urate
- Long periods of active, untreated gout

GOUT RISK FACTORS

- Male
- Postmenopausal female
- Older
- Hypertension
- Pharmaceuticals:

  Diuretics, ASA, cyclosporine

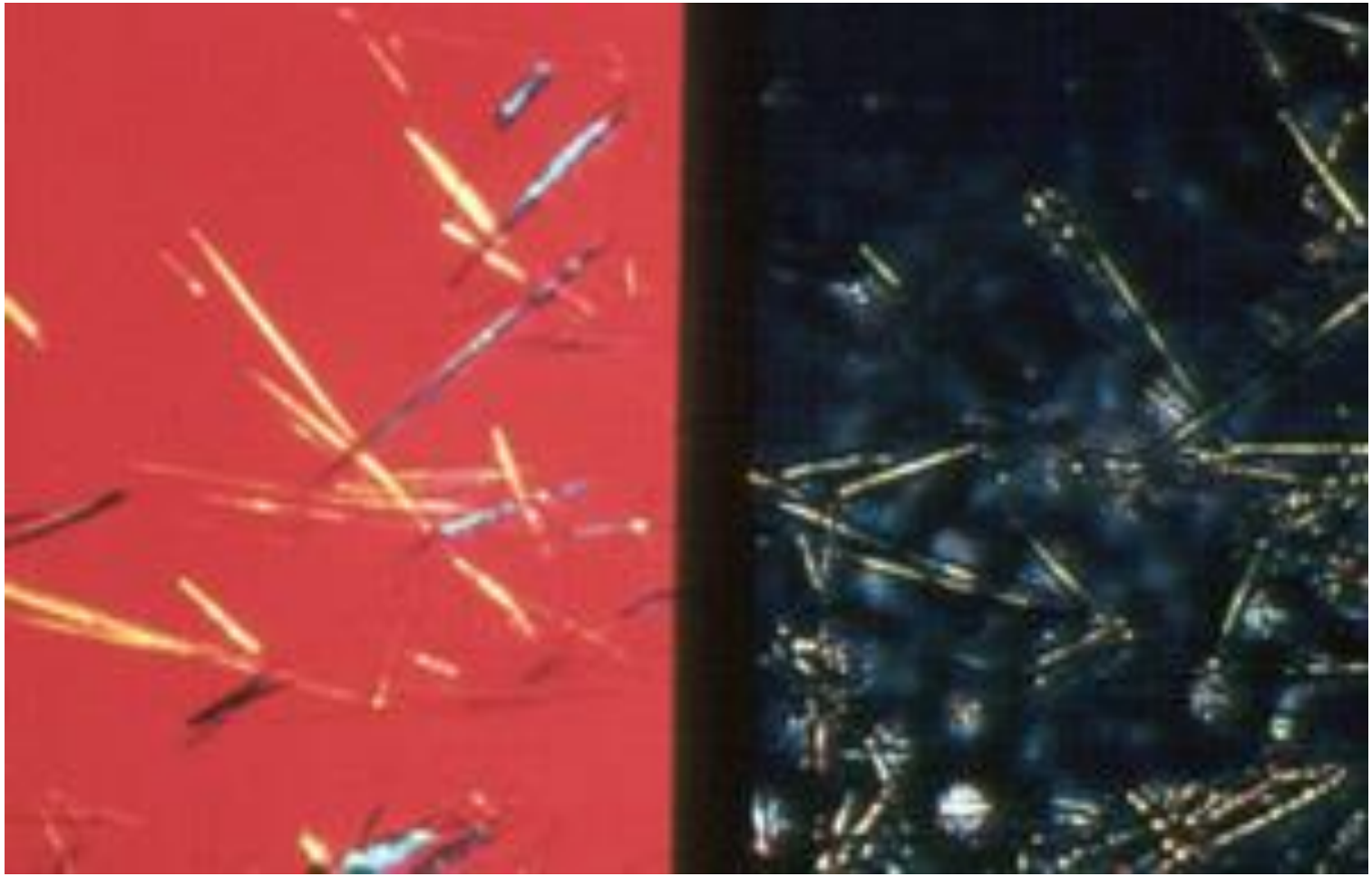
GOUT RISK FACTORS

- Transplant
- Alcohol intake

  Highest with beer

  High BMI (obesity)
- Diet high in meat & seafood

SYNOVIAL FLUID



URICOSURIC AGENTS

- Probenecid, (Losartan & fenofibrate for mild disease)
- Increased secretion of urate into urine
- Reverses most common physiologic abnormality in gout (90% pt.s are underexcretors)

Acute Flare Med Choices

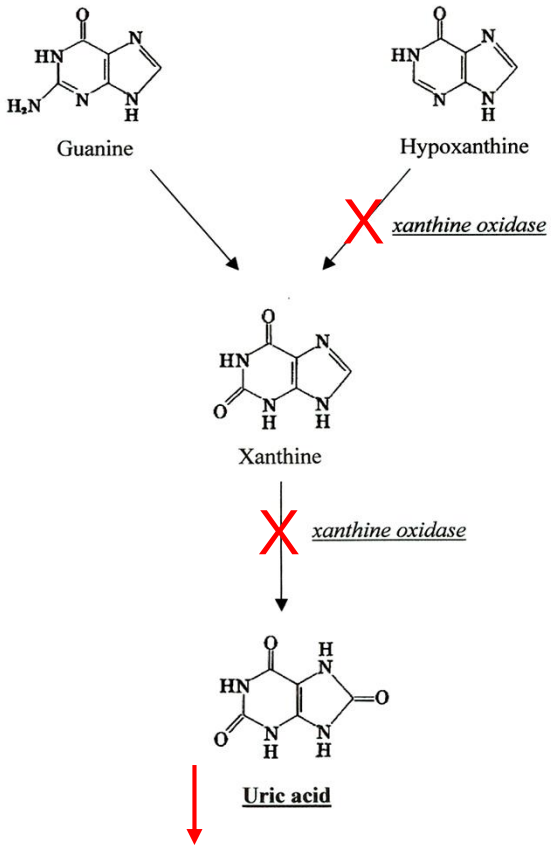
- NSAIDS
- Colchicine
- Corticosteroids

XANTHINE OXIDASE INHIBITOR

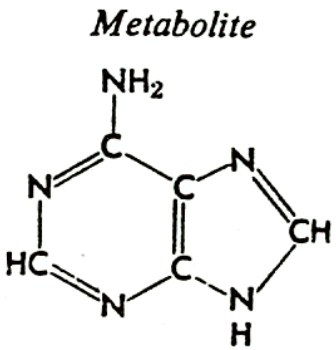
- Allopurinol :
- Blocks conversion of hypoxanthine to uric acid
- Effective in overproducers
- May be effective in underexcretors
- Can work in pt.s with renal insufficiency

Allopurinol

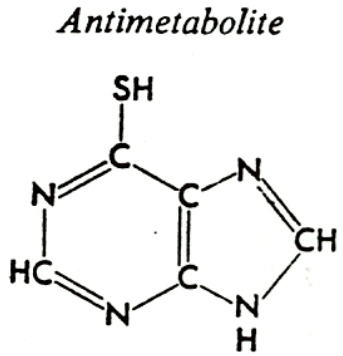
Inhibits xanthine oxidase



Inhibition of Purine Biosynthesis by the Antitumor Agent, 6-Mercaptopurine



Adenine



6-Mercaptopurine

1) 6-Mercaptopurine is converted to a nucleotide.

2) The nucleotide <u>inhibits purine biosynthesis</u> at steps 2, 12,, and 13.

What is LNS?

- LNS is a genetic disorder first discovered in 1964 by Michael Lesch and William Nyhan.
- It is a recessive disease that is linked to the X chromosome
- It is caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT)

HPRT's role in the body

- Hypoxanthine-guanine phosphoribosyltransferase is an enzyme that plays a key role in the recycling of the purine bases, hypoxanthine, and guanine into purine nucleotide pools
- Without HPRT the purine bases are broken down and excreted as uric acid
- Since these purine bases cannot be reused, the production of purine bases is increased
- Both of these together cause a significant overproduction of uric acid

Symptoms of LNS

All of the following are a result of an overproduction of Uric Acid

- Urate crystal formations, which look like orange sand, are deposited in diapers of the babies
- Kidney stones
- Blood in the urine
- Dysphagia (difficulty swallowing)
- Swelling of the joints
- Vomiting
- Athetosis (uncontrolled spastic muscle movements of the arms and legs)
- Chorea (purposeless repetitive movements)
- Moderate mental retardation
- Irritability

Statistics

- **Frequency**:

  -Reported prevalence is 1 per 380,000

- **Mortality**:

  -Few patients live beyond 40 years.

  -The drug allopurinol is used to control hyperuricernia. Most patients experience progressive dysphagia (difficulty swallowing) and die after pneumonia .

  -Also common is sudden unexpected death, even to patients in stable medical condition.

- **Race**:

  -LNS effects most races with equal frequency.

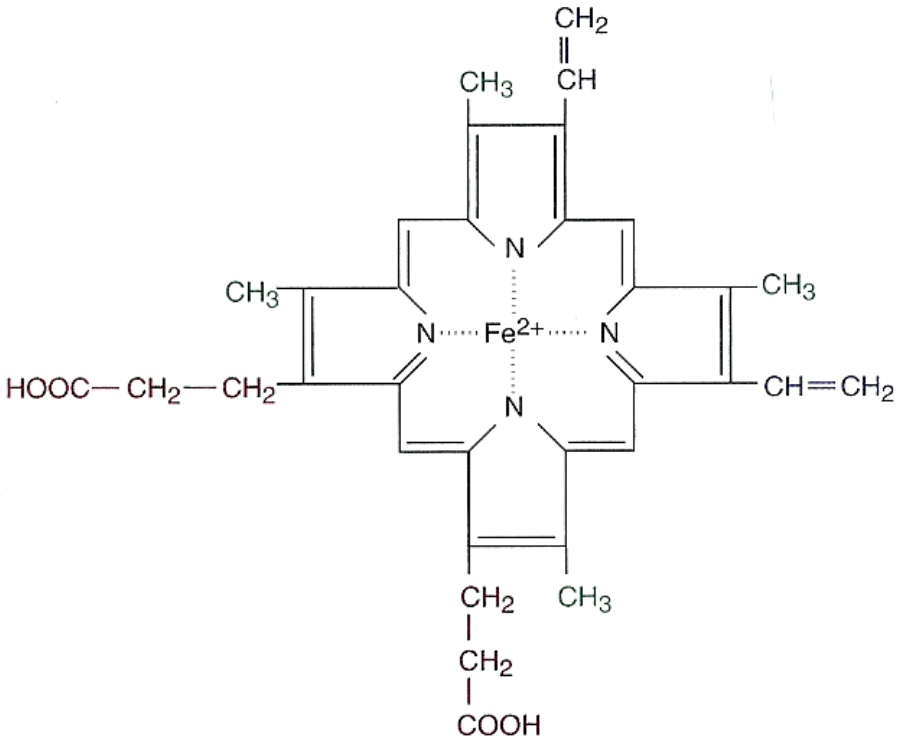
Heme

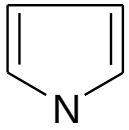
Porphyrines

Overview of Porphyrias

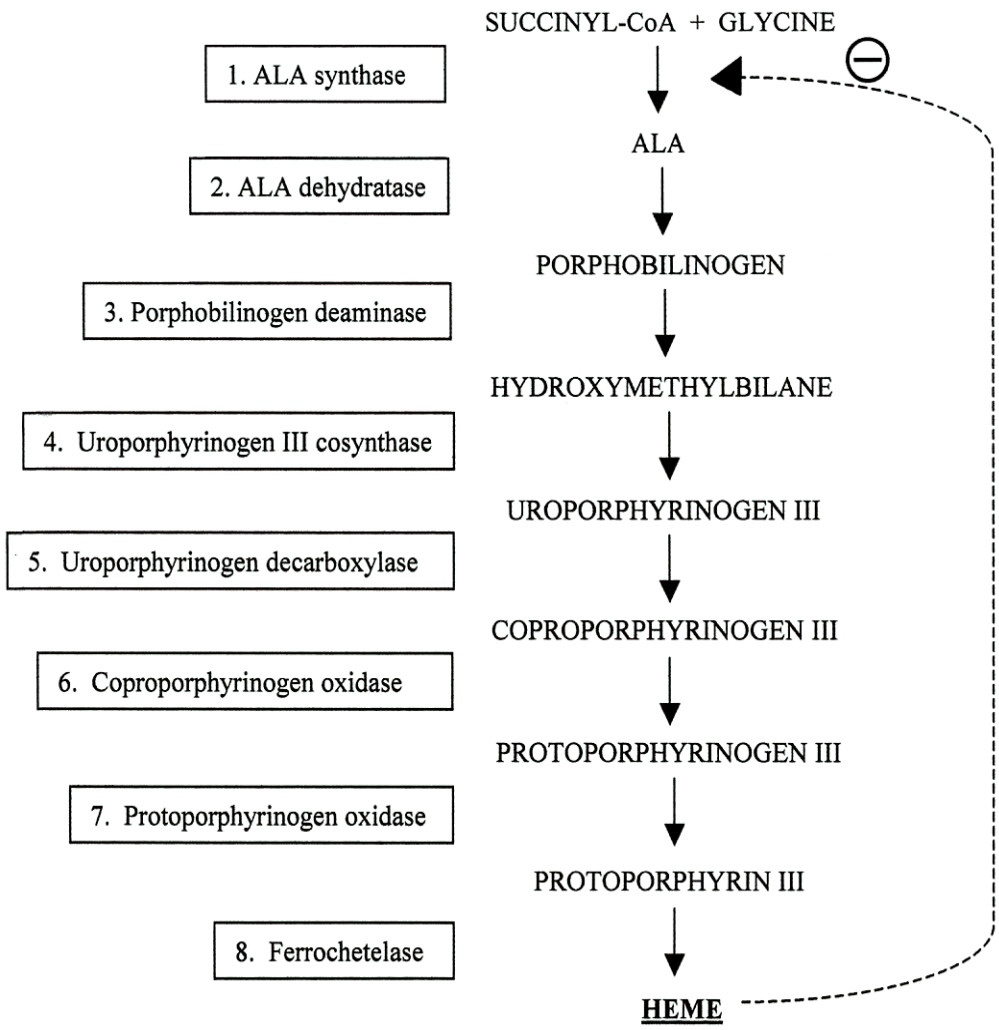
- Metabolic disorders involving defects in heme biosynthesis
- May be inherited or acquired
- Most inherited forms are autosomal dominant, some are autosomal recessive

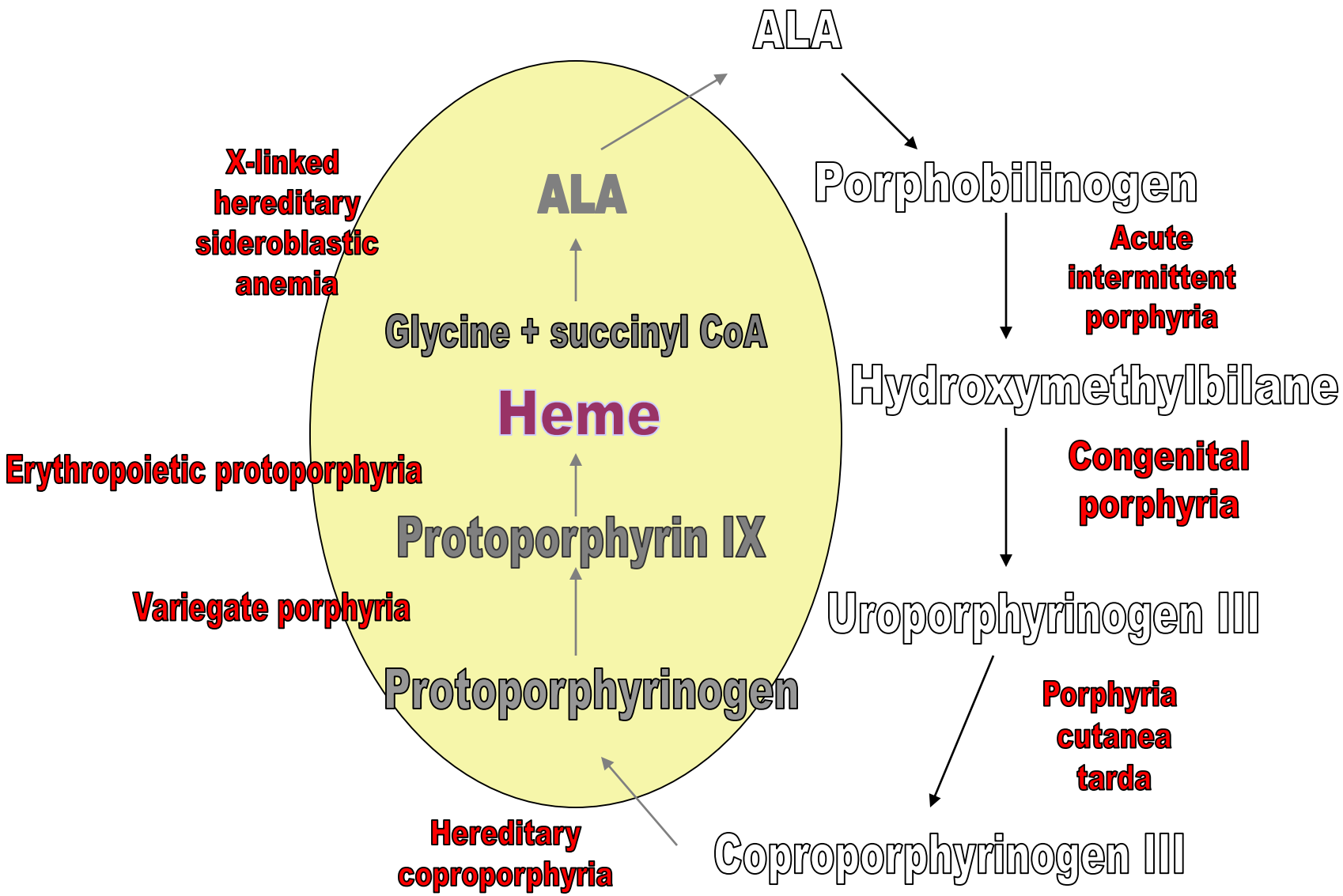
Structure





Pyrrole


SUCCINYL-CoA + GLYCINE
1. ALA synthase
ALA
2. ALA dehydratase
PORPHOBILINOGEN
3. Porphobilinogen deaminase
HYDROXYMETHYLBILANE
4. Uroporphyrinogen III cosynthase
UROPORPHYRINOGEN III
5. Uroporphyrinogen decarboxylase
COPROPORPHYRINOGEN III
6. Coproporphyrinogen oxidase
PROTOPORPHYRINOGEN III
7. Protoporphyrinogen oxidase
PROTOPORPHYRIN III
8. Ferrochetelase
HEME



ALA = 5-aminolevulinate

Porphyrias

Disease	*Enzyme Deficiency (#)*	*Genetics*	*Pathology*
More common:			
Acute intermittent porphyria	Porphobilinogen deaminase **(3)**	Dominant	Nervous system
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase **(5)**	Dominant	Skin
Erythropoietic protoporphyria	Ferrochetalase **(8)**	Dominant	Skin, gallstones, liver disease
Less common:			
Congenital erythropoietic porphyria	Uroporphyrinogen III cosynthase **(4)**	Recessive	Skin, appendages, reticuloendothelial system
Hereditary coproporphyria	Coproporphyrinogen oxidase **(6)**	Dominant	Nervous system, skin
Variegate porphyria	Protoporphyrinogen Oxidase **(7)**	Dominant	Nervous system, skin

Overview of Porphyrias:

- Classified into acute and non-acute
- Acute:
  - Acute intermittent porphyria
  - Variegate porphyria
  - Hereditary coproporphyria
- Non-acute:
  - Porphyria cutanea tarda
  - Erythropoietic protoporphyria
  - Congenital Porphyria

Porphyrinurias: lead, alcohol, iron-deficiency anemia, liver disease

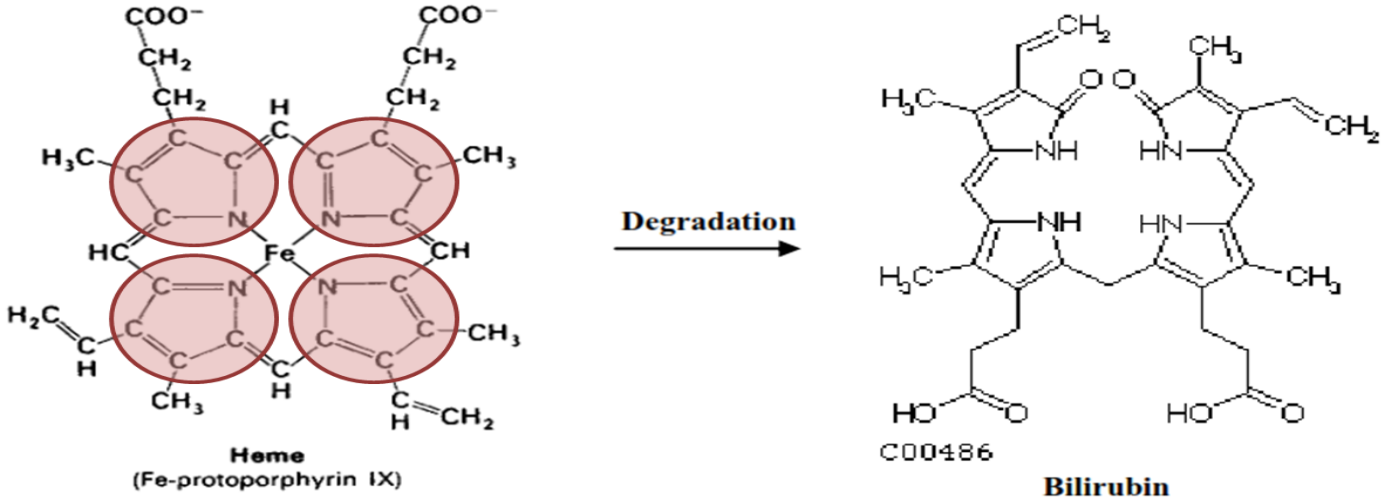
Erythropoietic Protoporphyria

Porphyria Cutanea Tarda

Hemoglobin Catabolism

Heme and bilirubin

- Heme four **pyrrols** rings connected together to form (porphyrin).
- Bilirubin consists of **open chain** of four **pyrrols-like** rings



Heme
(Fe-protoporphyrin IX)

Bilirubin

Definition of bilirubin

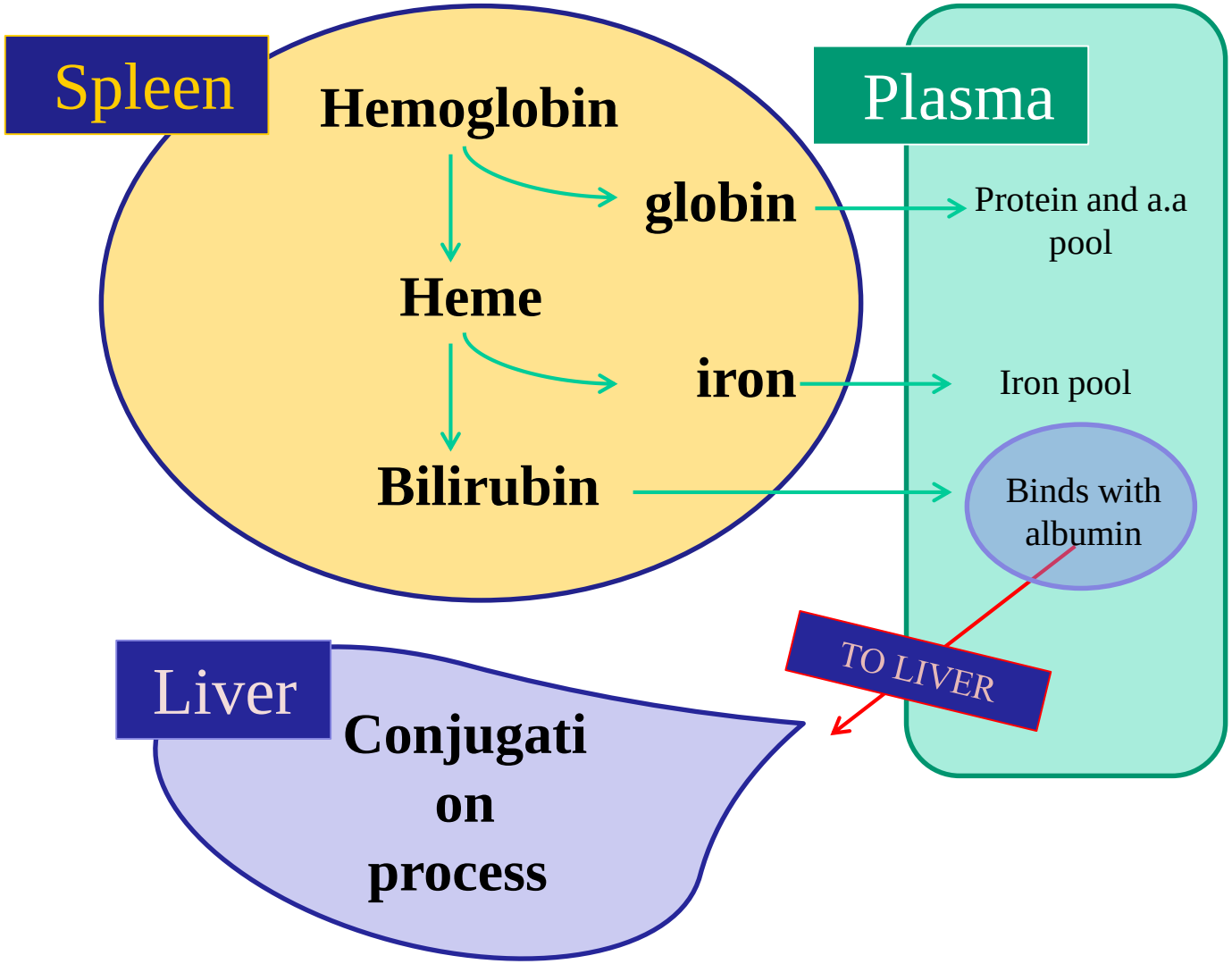
- Bilirubin is the water insoluble breakdown product of normal heme catabolism
- It's a yellow pigment present in bile (a fluid made by the liver) , urine and feces .
- Heme is found in hemoglobin, a principal component of RBCs [Heme: iron + organic compound "porphyrin"].

❖ Heme source in body:

➢ 80% from hemoglobin

➢ 20% other hemo-protein: cytochrome, myoglobin)

Hemoglobin degrading and bilirubin formation



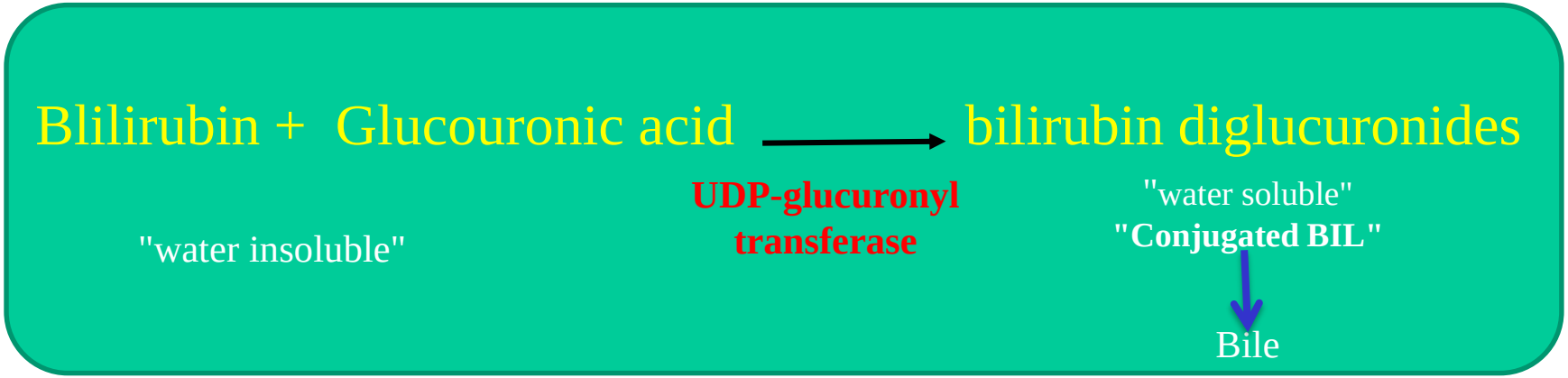
Bilirubin Metabolism

1. **Unconjugation process :**

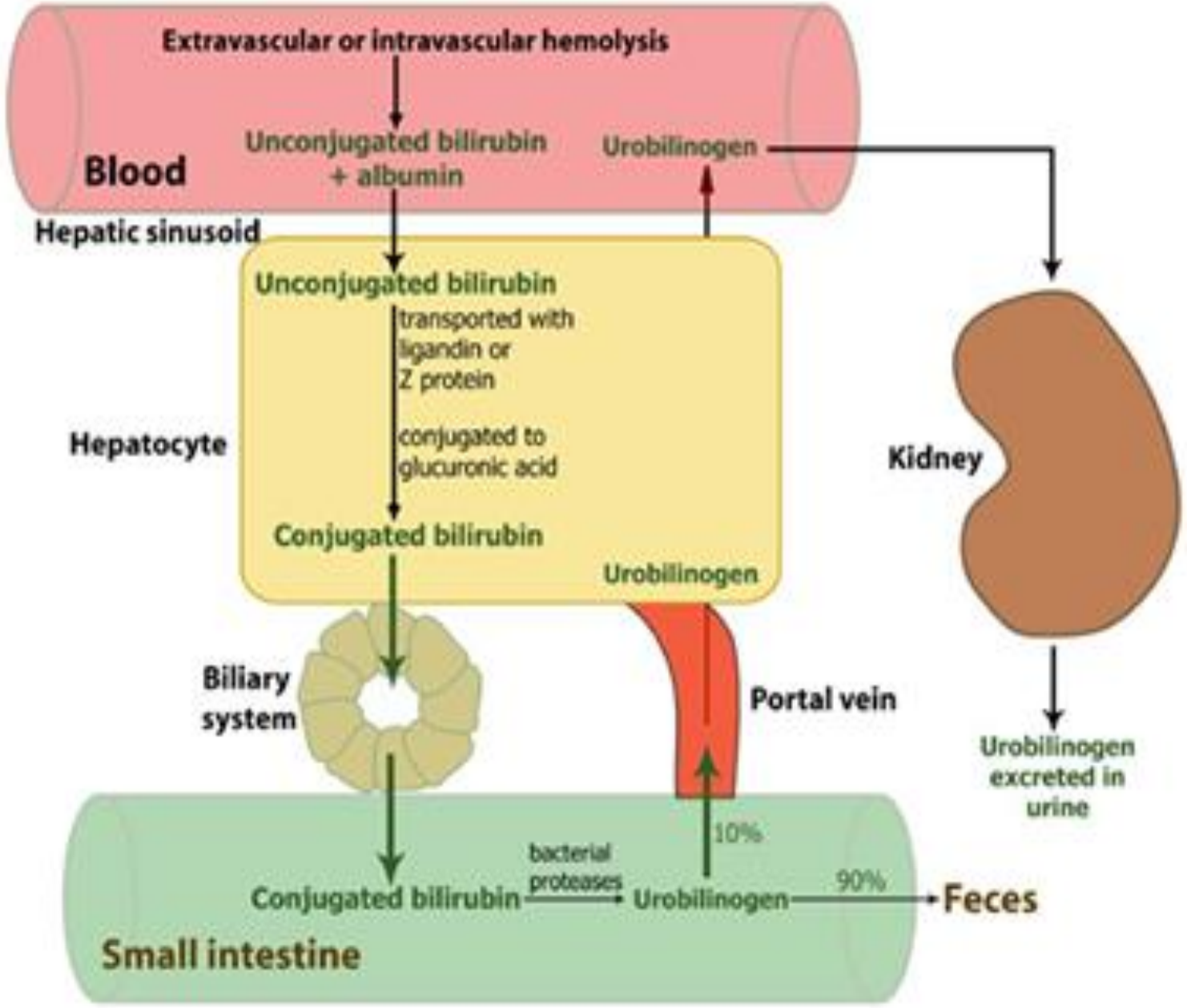
- RBCs are phagocytized in the **spleen**. Hemoglobin is catabolized into amino acids, iron and **heme**.
- Heme ring is broken open and converted to **unconjugated (indirect) bilirubin.**
- This unconjugated bilirubin is not soluble in water, due to intramolecular hydrogen bonding. It is then bound to albumin and sent to the liver.

2. Congugation process:

- **In liver:** Bilirubin is **conjugated** with Glucouronic acid to produce bilirubin diglucuronides, which is water soluble and readily transported to bile. and thus out into the small intestine.

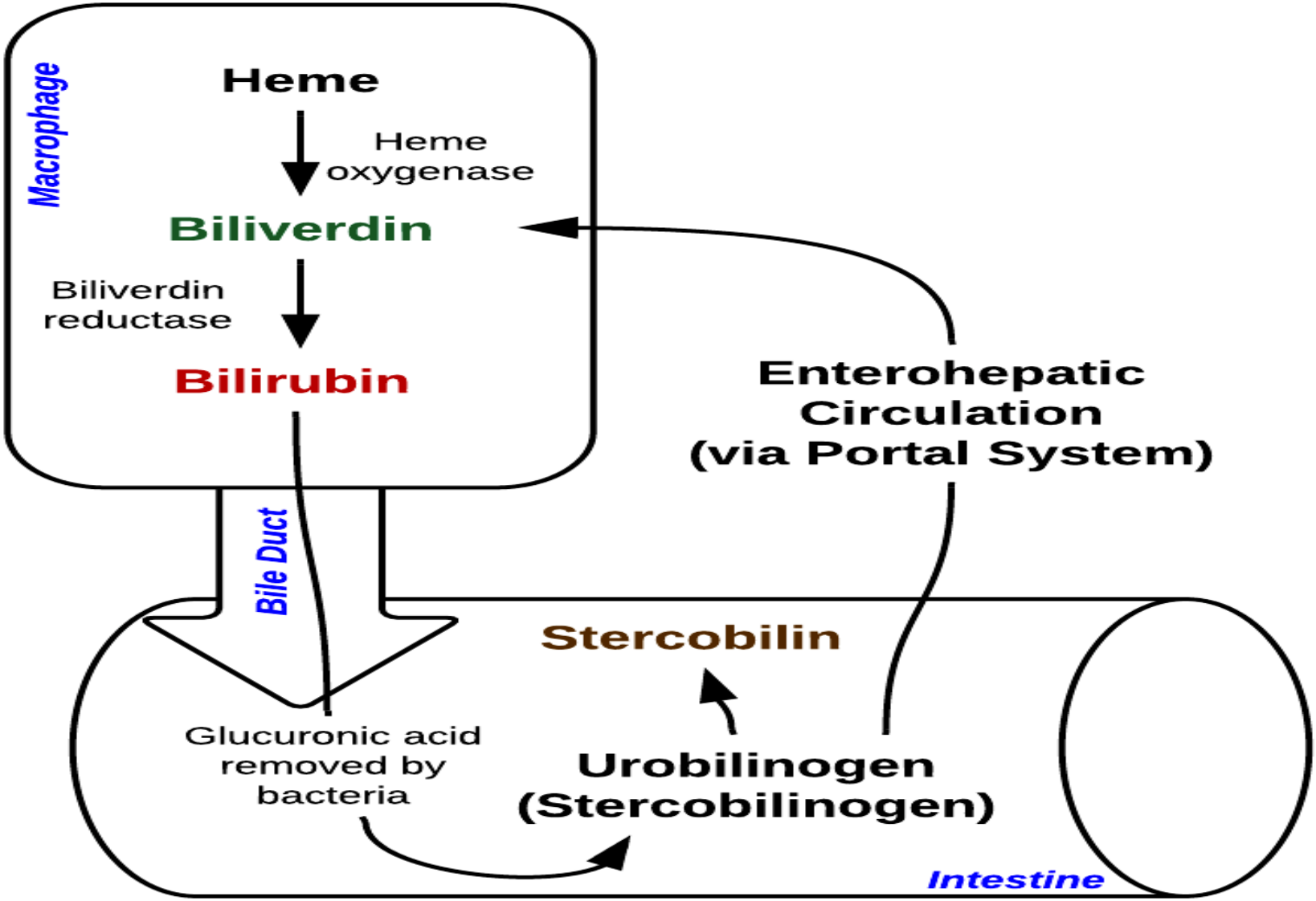


- Then conjugated bilirubin is excreted in bile through bile duct to help in **food digestion (mainly fat)**.


Extravascular or intravascular hemolysis
Blood
Unconjugated bilirubin
+ albumin
Urobilinogen
Hepatic sinusoid
Unconjugated bilirubin
transported with
ligandin or
Z protein
Hepatocyte
conjugated to
glucuronic acid
Conjugated bilirubin
Urobilinogen
Kidney
Biliary
system
Portal vein
Urobilinogen
excreted in
urine
10%
bacterial
proteases
Conjugated bilirubin
Urobilinogen
90%
Feces
Small intestine

- The excess amount transferred to **intestine** to be excreted in urine and stool.
- ❖ However 95% of the secreted bile is reabsorbed by the small intestine. This bile is then resecreted by the liver into the small intestine. This process is known as enterohepatic circulation
- About half of the conjugated bilirubin remaining in the large intestine (about 5% of what was originally secreted) is metabolised by colonic bacteria to form urobilinogen , which may be further oxidized to urobilin and stercobilin . Urobilin, stercobilin and their degradation products give feces its brown color.

- Elevated levels of bilirubin in blood and urine indicate certain diseases.


Macrophage
Heme
Heme oxygenase
Biliverdin
Biliverdin reductase
Bilirubin
Enterohepatic Circulation (via Portal System)
Bile Duct
Stercobilin
Glucuronic acid removed by bacteria
Urobilinogen (Stercobilinogen)
Intestine

- ✓ Direct bilirubin: is conjugated (water soluble bilirubin) in aqueous solution it reacts rapidly with reagent (direct reacting).

- ✓ Indirect bilirubin: is unconjugated (water insoluble bilirubin) because it is less soluble in it reacts more slowly with reagent (reaction carried out in methanol).

  - in this case both conjugated and unconjugated bilirubin are measured given **total bilirubin**. Unconjugated will calculated by subtracting direct from total and so called indirect.

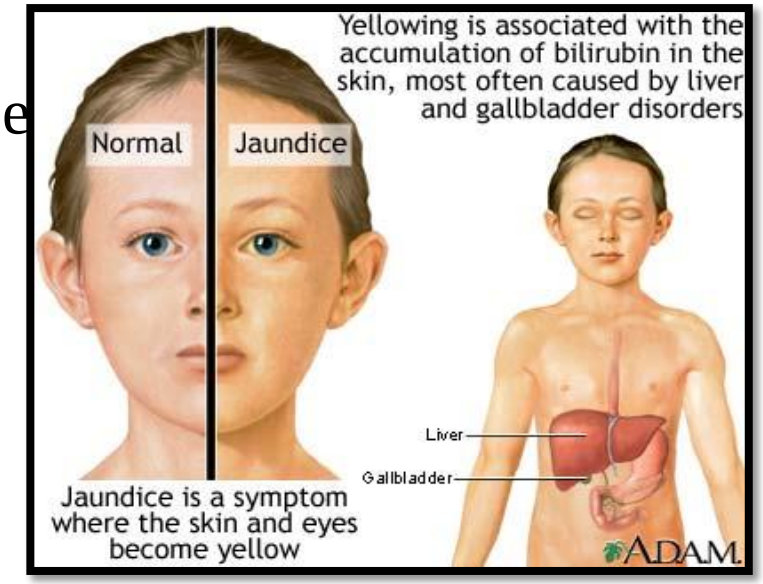
- ✓ Total bilirubin = D+ ID

- **Knowing the level of each type of bilirubin has diagnostic important.**

- It is a medical term describes the elevation of bilirubin in blood result in yellow color of skin and sclera.(2-2.5mg/dl)
- Other symptoms include nausea, vomiting, dark-colored urine , fatigue
- **Types of Jaundice:**
- according to the cause of jaundice

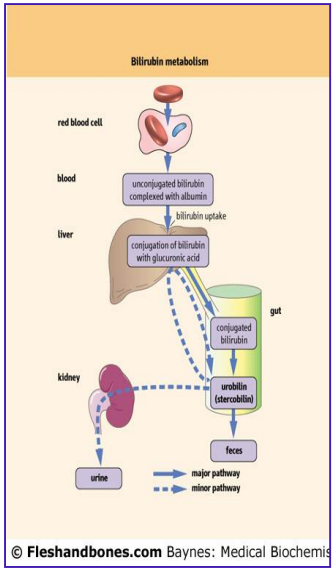
it is classified to three main types:

- ❖ Pre-hepatic jaundice
- ❖ Hepatic jaundice
- ❖ Post-hepatic (most common type)



HYPERBILIRUBINEMIA

- **Increased plasma concentrations of bilirubin (> 3 mg/dL) occurs when there is an imbalance between its production and excretion**
- **Recognized clinically as jaundice**



The causes of jaundice

Type	Cause	Clinical example	Frequency
Prehepatic	hemolysis	autoimmune abnormal hemoglobin	uncommon depends on region
intrahepatic	infection	hepatitis A, B, C	common/very common
	chemical/drug	acetaminophen alcohol	common common
	genetic errors: bilirubin metabolism	Gilbert's syndrome Crigler–Najjar syndrome Dubin–Johnson syndrome Rotor's syndrome	1 in 20 very rare very rare very rare
	genetic errors: specific proteins	Wilson's disease α_1 antitrypsin	1 in 200 000 1 in 1000 with genotype
	autoimmune	chronic active hepatitis	uncommon/ rare
	neonatal	physiologic	very common
Posthepatic	intrahepatic bile ducts	drugs primary bilary cirrhosis cholangitis	common uncommon common
	extrahepatic bile ducts	gall stones pancreatic tumor cholangiocarcinoma	very common uncommon rare

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	haemolytic jaundice **Pre-hepatic jaundice**	hepato-cellular jaundice **Hepatic jaundice**	obstructive jaundice **Post-hepatic jaundice**
Causes	➢ Due to increase in RBCs breakdown due to hemolytic anemia. ➢ The rate of RBCs lysis and bilirubin production more than ability of liver to convert it to the conjugated form ❑ Occur in: ➢Erythroblastosis fetalis ➢Hemolytic anemia ➢Transfusion reaction	➢ Due to liver cell damage (cancer, cirrhosis or hepatitis) ➢ Conjugation of bilirubin decreased (ID.Bil. ↑). ➢ Blilirubin that is conjugated is not efficiently secreted into bile but leaks to blood (D.Bil. ↑) ❑ Occur in : ➢Cirrhosis (scarring of the liver) ➢Hepatitis ➢Gilbert's disease	➢ Due to obstruction of bile duct which prevents passage of bilirubin into intestine. ➢ D.Bil will back to liver and then to circulation elevating its level in blood and urine. ❑ Occur in: ➢Biliary stricture ➢Cancer of the pancreas or gallbladder ➢Gallstones
Type of Bil.	ID.Bil > D.Bil	D.Bil, ID.Bil, T.Bil all (High)	D.Bil (High)
Conformational test	K^+ (High) Hematology: CBC (low Hb)	ALT, AST (High)	ALP (High)

Physiologic Jaundice of the newborn:

- High bilirubin levels is common

- After birth the newborns breaking down the excess RBCs they are born with and, because the newborn's liver is not fully mature, (unable to process the extra bilirubin) leads to elevate its level in blood and other body tissues.

SO, WHAT TYPE OF JUNDUCE IS THIS ???

- ❑ Common, particularly in premature infants
- ❑ Transient (resolves in the first 10 days)
- ❑ Due to immaturity of the liver enzymes
- ❑ Jaundice within the first 24 hrs of life or which takes longer then 10 days to resolve is usually pathological and needs to be further investigated
- ❑ High levels of unconjugated bilirubin are toxic to the newborn – ca type of mental retardation known as kernicterus

❖ New born jaundice treatment:

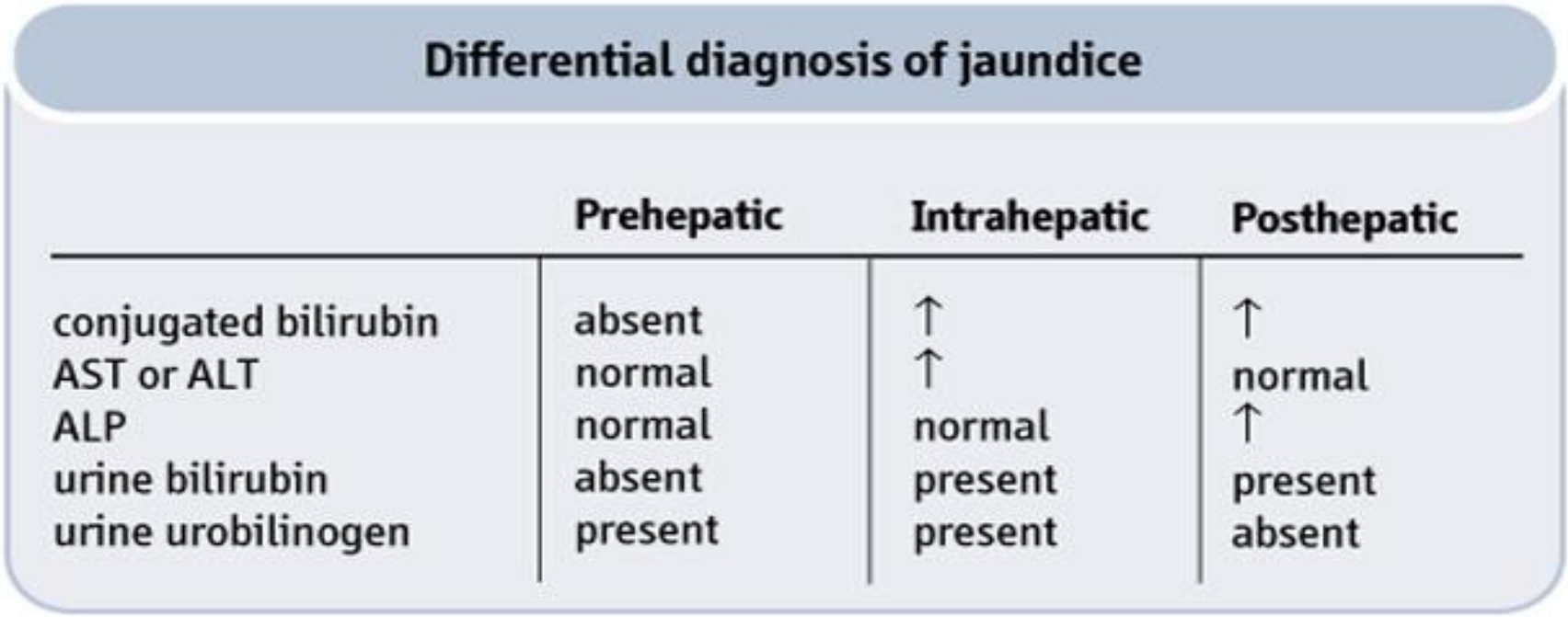
- Usually newborn is treated by **phototherapy** which breakdown bilirubin (ID→D) and convert it to the photo isomer form which is more soluble.

Bilirubin Toxicity :

❖ **Very high bilirubin is danger and toxic it may cause**

➢ brain damage ⟶ effect on muscles, eyes and Leading to death

Diagnoses of Jaundice



Differential diagnosis of jaundice

	Prehepatic	Intrahepatic	Posthepatic
conjugated bilirubin	absent	↑	↑
AST or ALT	normal	↑	normal
ALP	normal	normal	↑
urine bilirubin	absent	present	present
urine urobilinogen	present	present	absent

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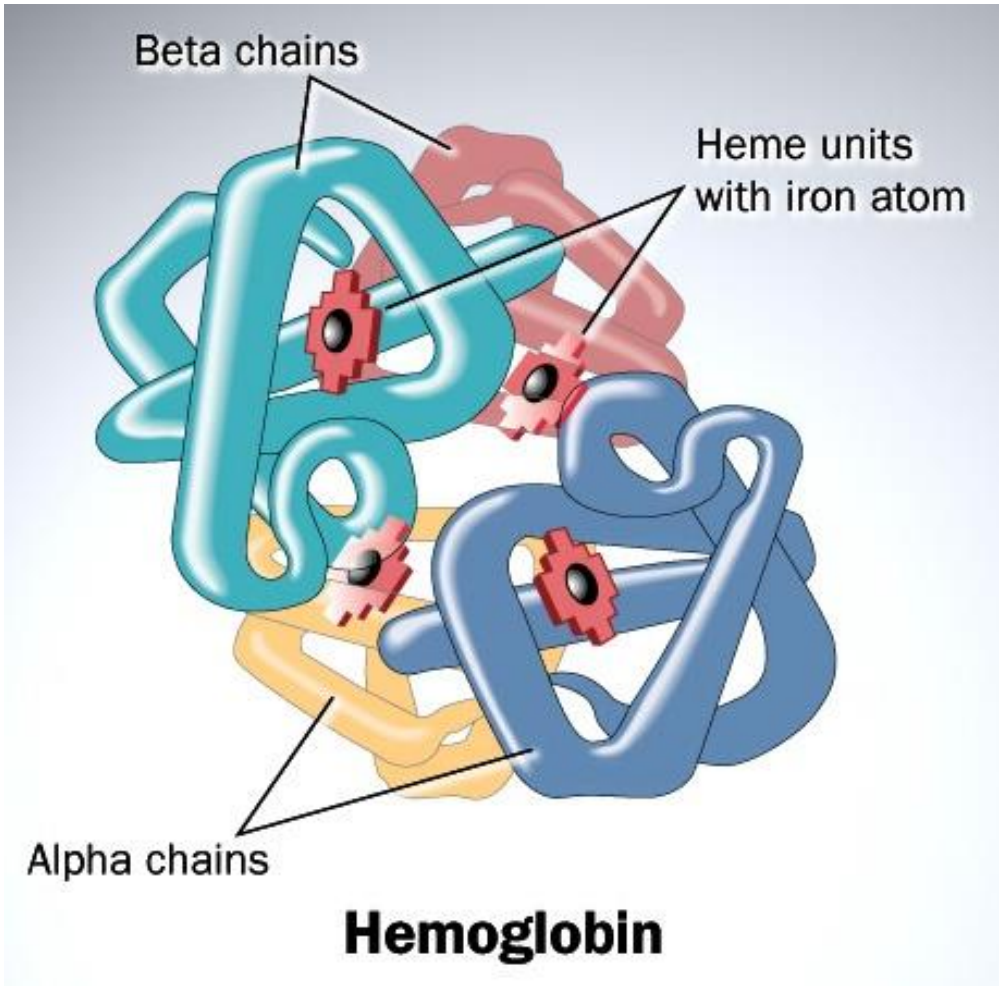
Hemoglobinopathies

- Decrease, lack of, or abnormal globin
- May be severe hemolytic anemia
- Abnormal Hb with low functionality
- Mutation may be deletion, substitution, elongation
- Hb electrophoresis may be helpful

Hb-A Molecule. Hb-A is the major adult hemoglobin.

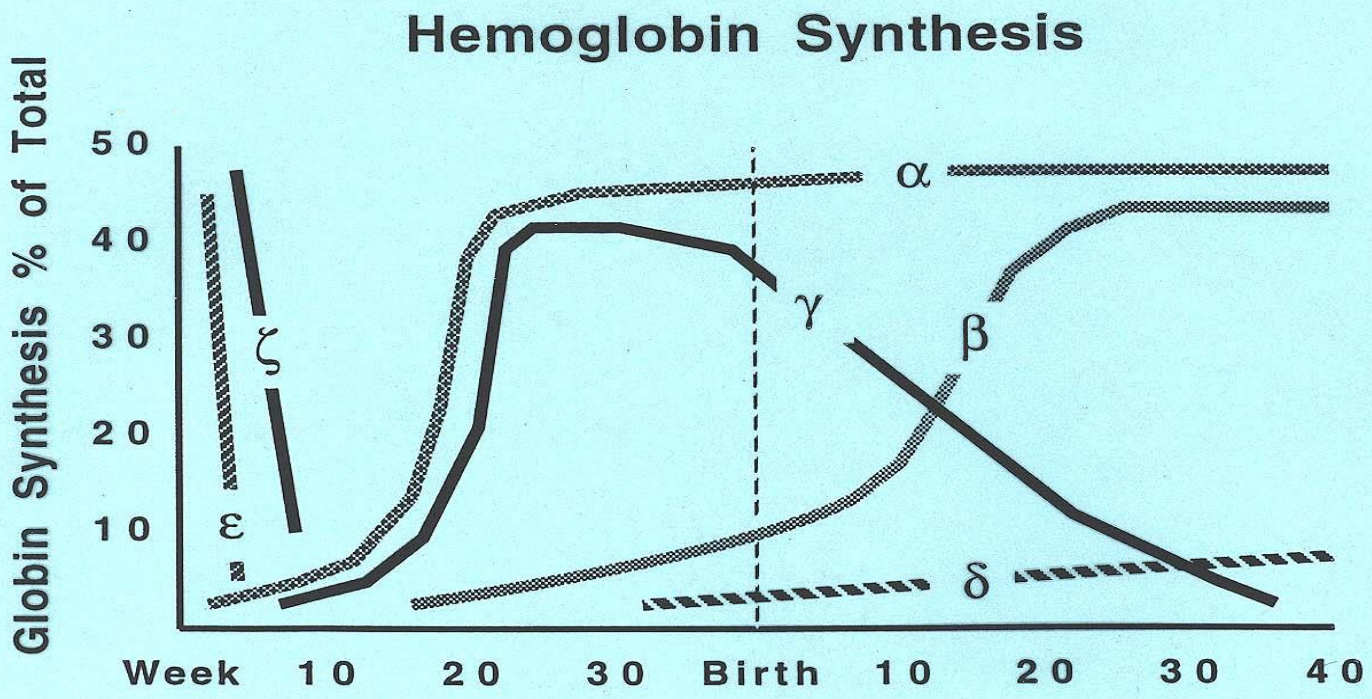


Hemoglobin

- Heme
  - Porphyrin ring and Fe
- Globins
  - Alpha family on chromosome 16

    ––[ζ]--//--[α]––
  - Beta family on chromosome 11

    ––[ε]--//--[γ]—[δ]––[β]––



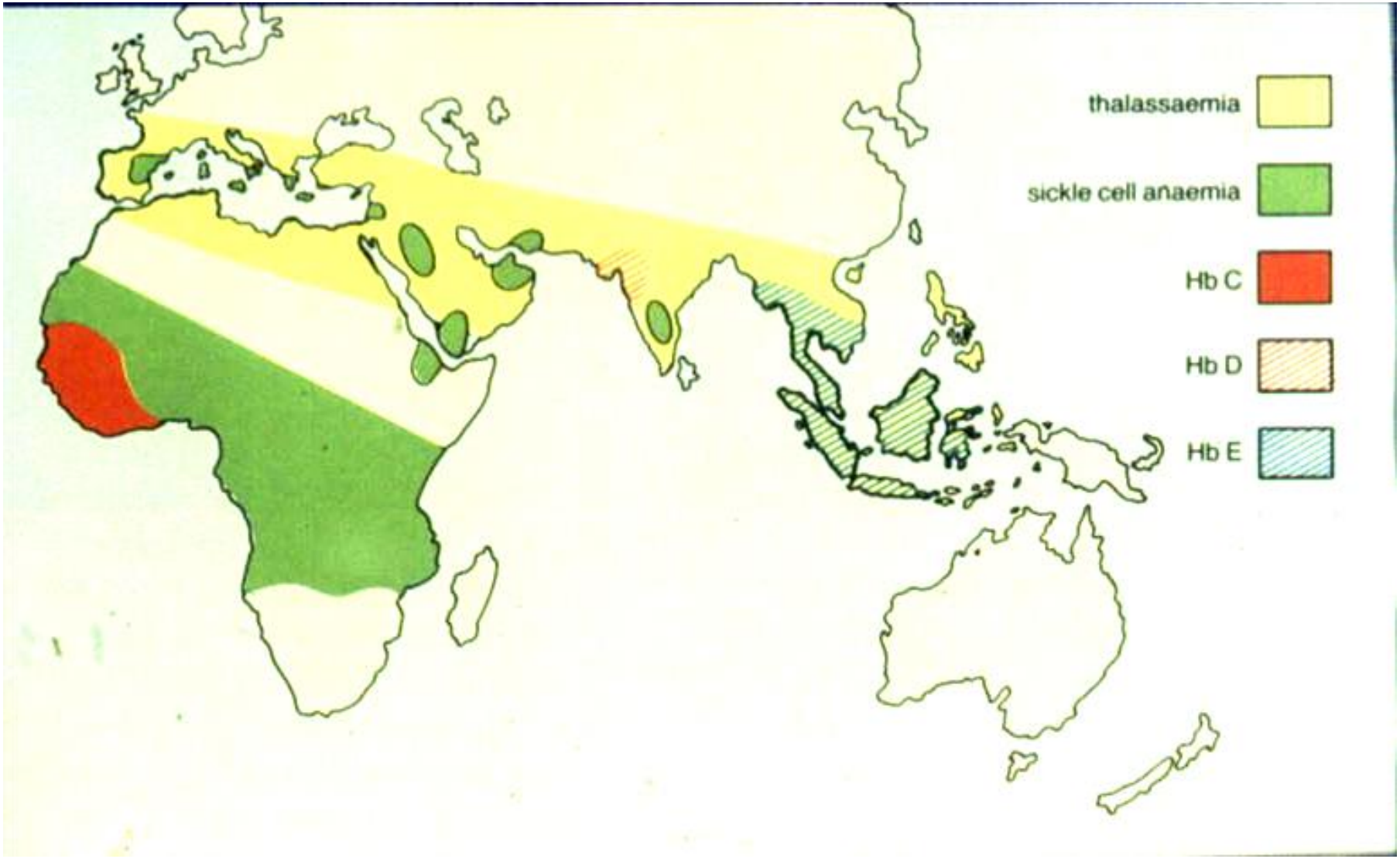
		Adult	Newborn
α2β2	Hb A	97 %	20 %
α2δ2	Hb A2	2.5	<0.5
α2γ2	Hb F	<1	80

Embryonic:

ζ2ε2	Gower-1
α2ε2	Gower-2
ζ2γ2	Portland

Thalassemia

- 1925: Described by Dr. Thomas Cooley and Dr. Pearl Lee of Detroit
- 1930's: Familial pattern recognized
- 1950's: Alkali denaturation test for Hb F, Hb ELP
- 1960's: RBC indices
- 1980's: Histogram, DNA analysis, PCR


thalassaemia
sickle cell anaemia
Hb C
Hb D
Hb E

How to name thalassemia?

- Named after globin chain that is abnormally synthesized !!!!

- Reduced or absent α-globin chain : α-thalassemia
- Reduced or absent β-globin chain : β-thalassemia
- Reduced or absent γ-globin chain : γ-thalassemia
- Reduced or absent δ-globin chain : δ-thalassemia
- Reduced or absent $\gamma\delta\beta$-globin chains
  : $\gamma\delta\beta$-thalassemia

Common types of thalassemia

- α-thalassemia
- β-thalassemia

ALPHA THALASSEMIAS

α Thalassemia

- Absence of α chains will result in increase/ excess of **γ** globin chains during fetal life and excess β globin chains later in postnatal life.
- Severity of disease depends on number of genes affected.

Symbolism
Alpha Thalassemia

(/) : Indicates division between genes inherited from both parents:

αα/αα (Normal)

- Each chromosome 16 carries 2 genes. Therefore the total complement of α genes in an individual is 4.

Symbolism
Alpha Thalassemia

(-) : Indicates a gene deletion:

$-\alpha/\alpha\alpha$

- α^+ Thalassemia (one gene deletion)
- 3 functional working genes.
- Called α thal 2.

Symbolism
Alpha Thalassemia

(-) : Indicates a gene deletion:

--/αα

- α^0 Thalassemia (two gene deletion) in the same chromosome.
- 2 functional working genes.
- Called α thal 1.

Symbolism
Other Thalassemia

- Superscript T denotes nonfunctioning (mutated gene, not deletion) gene:

- α^{T}

Classification & Terminology

Alpha Thalassemia

- Normal $\alpha\alpha/\alpha\alpha$
- Silent carrier - $\alpha/\alpha\alpha$
- Minor $-\alpha/-\alpha$

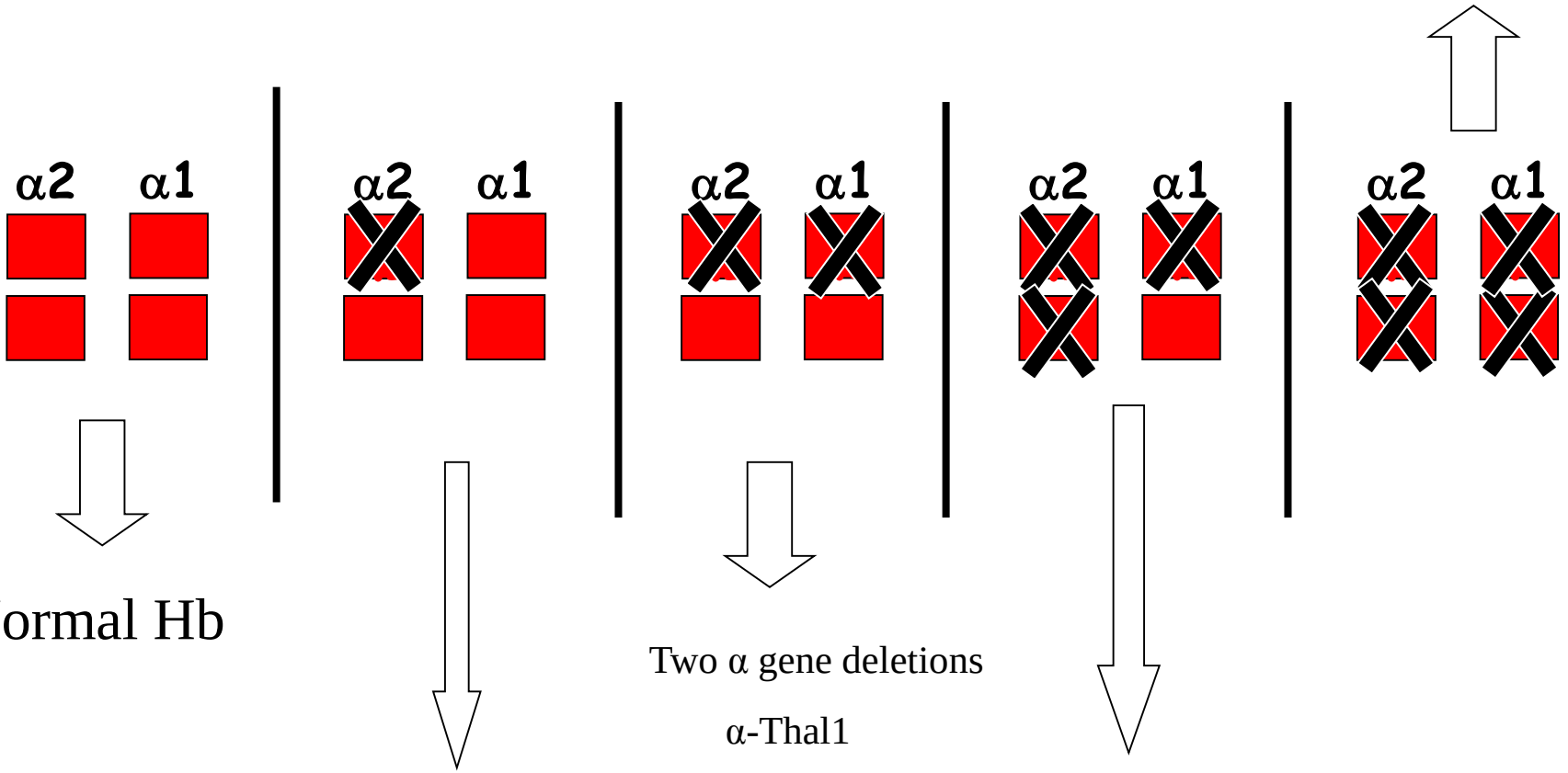
  $--/\alpha\alpha$
- Hb H disease $--/-\alpha$
- Barts hydrops fetalis $--/--$

α Thalassemia

Four α gene deletions

Hydrops fetalis or also called:

Erythroblastosis Fetalis.



Normal Hb

One α gene deletion

α-Thal2

Two α gene deletions

α-Thal1

Three α gene deletions

Hb-H disease

α Thalassemia

- As said, the genetic basis of α thal is mostly deletions: If you have 4 functional α genes, then you are normal.
- With 3 functional α genes, you are a silent carrier.
- With 2 functional α genes you have α thalassemia trait which is clinically benign, but there is mild microcytic anemia.
- With only one functional α chain, you have severe hemolytic anemia with primarily HbH, composed of 4 β chains (β4). This is clinically severe.
- In the absence of α chain in the fetus, the gamma forms a tetramer of globin chains, and is called Hb Bart's.
- Both Hb-H and Hb-Barts are high affinity Hbs, thus neither of them is capable of releasing oxygen to the tissues, also these hemoglobins are fast moving hemoglobins in Hb electrophoresis at alkaline pH.

α Thalassemia

- Infants with severe α Thalassemia (zero functional alpha genes) and Hb Barts suffer from severe intrauterine hypoxia and are born with massive generalized fluid accumulation, a condition known as hydrops fetalis or also called erythroblastosis fetalis.

Thus: in α Thalassemia

- Is usually caused by deletion of 1 or more of the 4 **α** globin genes on chromosome 16
- Severity of disease depends on number of the deleted α genes.
- Absence of α chains will result in increase/ excess of **γ** chains during fetal life and excess β chains later in life; Causes hemoglobins like Hb Bart's (γ_4) or HbH (β_4), to form which are physiologically useless (very high affinity).
- Like β thalassemia the excess globin chains causes the problem.

But:

- Alpha chain accumulation and deposition are more toxic than beta chain accumulation and deposition. Thus beta thalassemia is more severe than alpha thalassemia.

α Thalassemia

- Predominant cause of alpha thalassemias is large number of gene deletions in the α-globin genes.
- There are four clinical syndromes present in alpha thalassemia:
  - ♫ Silent Carrier State
  - ♫ Alpha Thalassemia Trait (Alpha Thalassemia Minor)
  - ♫ Hemoglobin H Disease
  - ♫ Bart's Hydrops Fetalis Syndrome

Silent Carrier α Thalassemia

- -α/αα
- One alpha gene deletion, 3 intact alpha genes.
- Healthy persons.
- Normal Hb and Hct
- No treatment
- Can only be detected by DNA studies.

Alpha Thalassemia Trait

- Also called Alpha Thalassemia Minor.
- Caused by two missing alpha genes. May be homozygous (-α/-α) or heterozygous (--/αα).
- Exhibits mild microcytic, hypochromic anemia.
- MCV between 70-75 fL.
- May be confused with iron deficiency anemia.
- Although some Bart's hemoglobin (γ_4) present at birth, but no Bart's hemoglobin present in adults.

Hemoglobin H Disease

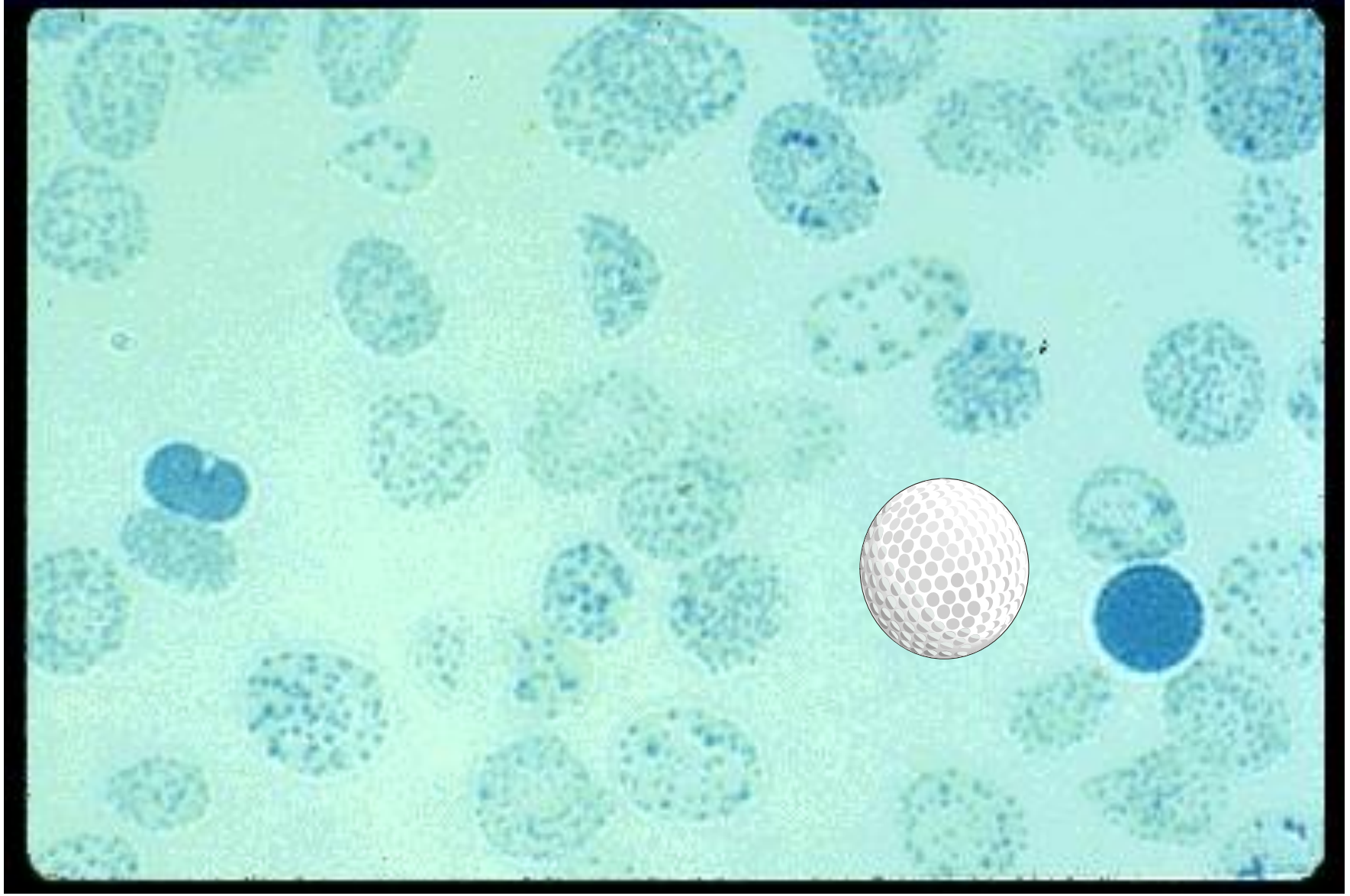
- Second most severe form alpha thalassemia.
- Usually caused by presence of **only one** intact α gene producing alpha chains (--/-**α**).
- Results in accumulation of excess unpaired gamma or beta chains. Born with 10-40% Bart's hemoglobin (**γ**4). Gradually replaced with Hemoglobin H (β4). In adult, have about 5-40% HbH.

γ4 ⟶ **β4**

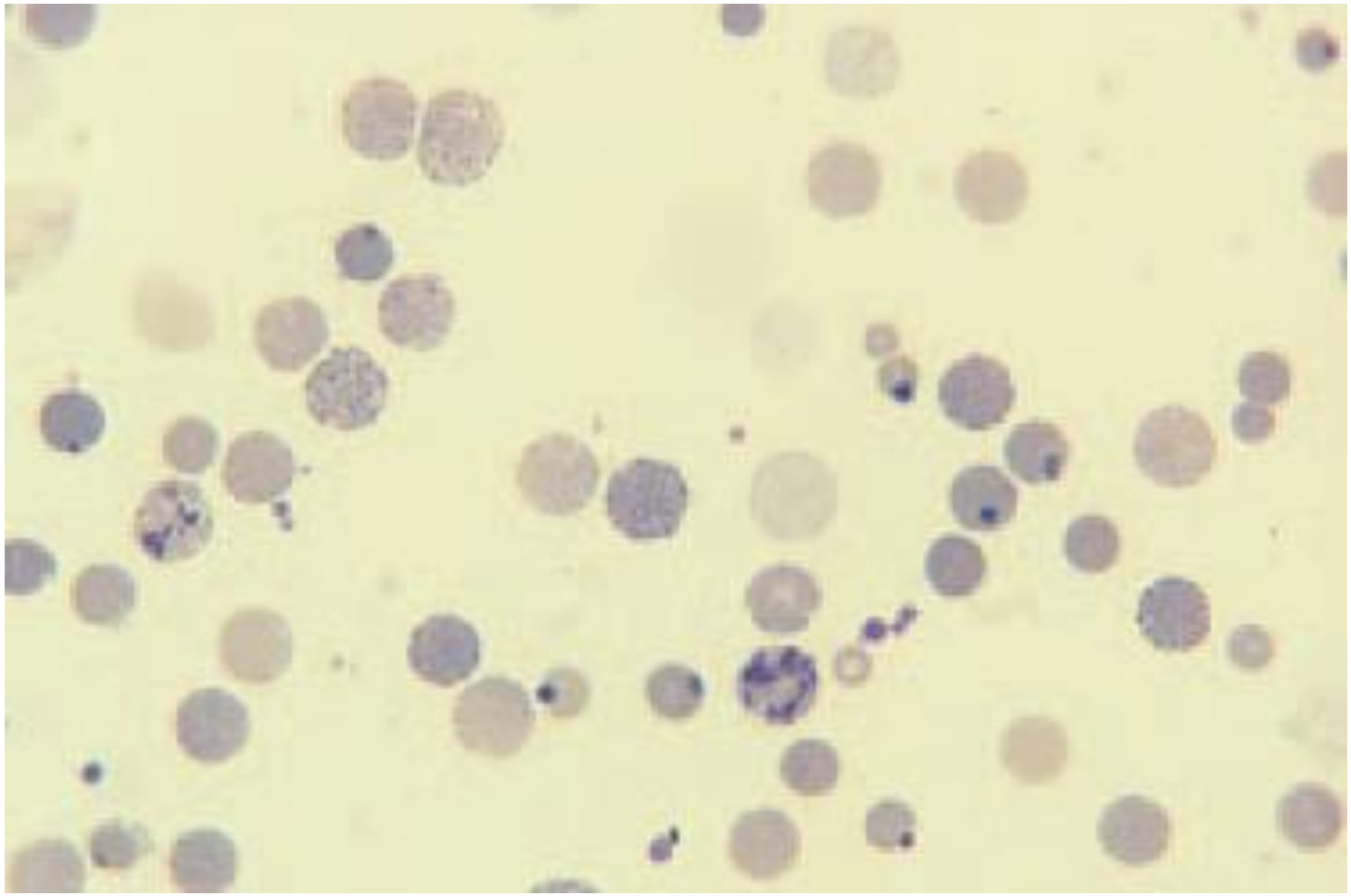
Hemoglobin H Disease

- Live normal life; however, infections, pregnancy, exposure to oxidative drugs may trigger hemolytic crisis.
- RBCs are microcytic, hypochromic with marked poikilocytosis. Numerous target cells.
- Hb 7-10 g/dl
- Hb electrophoresis: Fast moving band correspondent to HbH.
- HbH vulnerable to oxidation. Gradually precipitate in vivo to form Heinz-like bodies of denatured hemoglobin. Cells been described has having "golf ball" appearance, especially when stained with Brilliant Cresyl Blue.

Hemoglobin H Golfballs



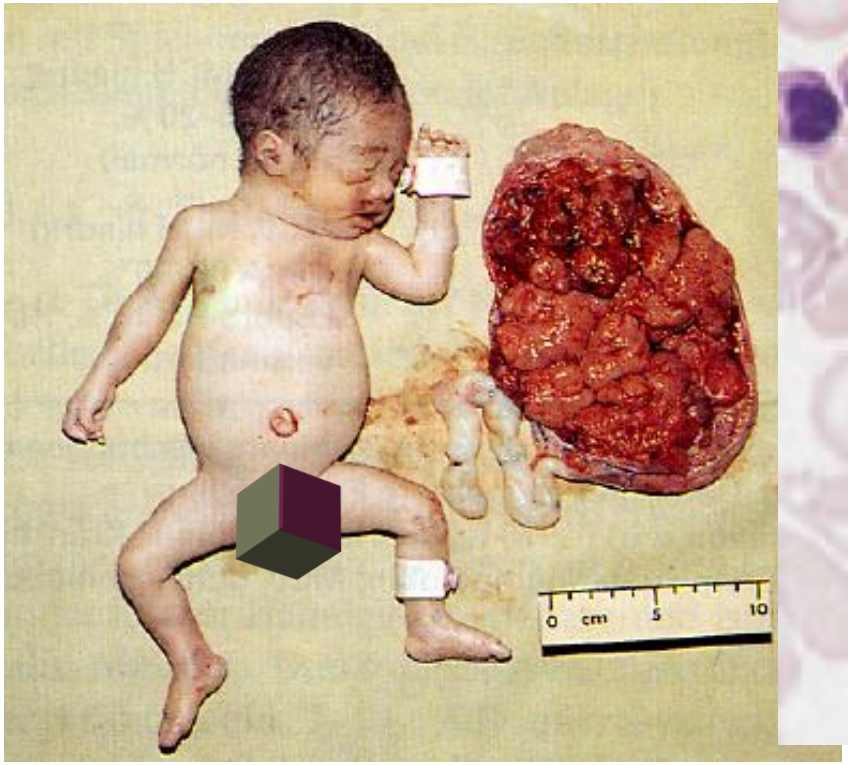
Hb-H inclusions

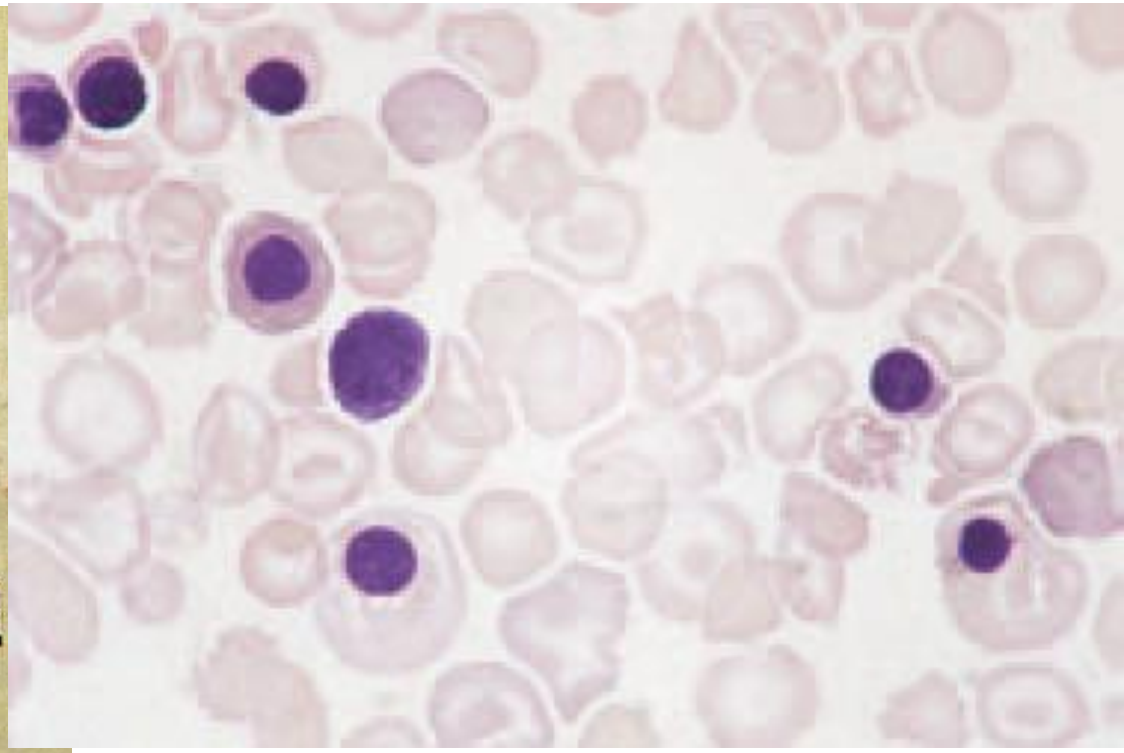


Bart's Hydrops Fetalis Syndrome

- Most severe form. Incompatible with life. Have no functioning α chain genes (- -/- -).
- Baby born with hydrops fetalis, which is edema and ascites caused by accumulation serous fluid in fetal tissues as result of severe anemia. Also we will see hepatosplenomegaly and cardiomegaly.
- Predominant Hb is Hb Bart, along with Hb Portland and traces of HbH.
- Hb Bart's has high oxygen affinity so cannot carry oxygen to tissues. Fetus dies in utero or shortly after birth. At birth, you will see severe hypochromic, microcytic anemia with numerous NRBCs.

Hydrops Fetalis





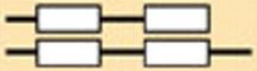
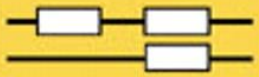
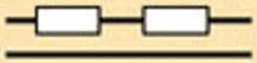
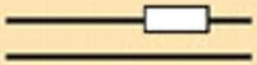
The blood film of neonate with hemoglobin Bart's hydrops fetalis showing anisocytosis, poikilocytosis and numerous nucleated red blood cells (NRBC).

State	Genotype	Genes	Features
Normal	αα/αα	4	normal
Hetero α^+ α-thal-2	αα/ – α	3	Essentially normal
Hetero α° α-thal-1	αα/ – –	2	Micro / Hypo Mild Anemia Bart's 2-8% (at birth) Hb H <2%
Homo α^+ α-thal-1	– α/ – α	2	
$\alpha^+ + \alpha^\circ$ Hb-H Disease	– α/ – –	1	Moderate Micro/Hypo anemia: Barts <10%, Hb H <40%
homo α° Hydrops	– – / – –	0	Hb A 0%, Bart's 70-80% Portland 10-20%

Comparison of α Thalassemias

Phenotype	Hb A	Hb Barts	Hb H
Normal	97-98%	0	0
Silent Carrier	96-98%	0-2% (At birth)	0
α Thalassemia Trait	85-95%	2-8% (At birth)	<2%
Hb H Disease	Dec	<10% (At birth)	5-40%
Hydrops Fetalis	0	70-80% (with 20% Hb Portland)	0-20%

α Thalassemia Syndromes

α-Thalassemia Syndromes		
α Gene Map	α Genotype	α Clinical Syndrome
	Normal	Normal
	Heterozygous α - Thal - 2 (α^{+} also called)	Silent Carrier of α Thalassemia
	Heterozygous α - Thal - 1 (α^{o} also called)	α - Thalassemia Trait
	Homozygous α - Thal - 2 (α^{+} also called homozygous)	α - Thalassemia Trait
	Compound Heterozygous α - Thal - 1 & 2 (α^{+}/α^{o} also called)	Hb - H Disease
	Homozygous α - Thal - 1	Hydrops Fetalis

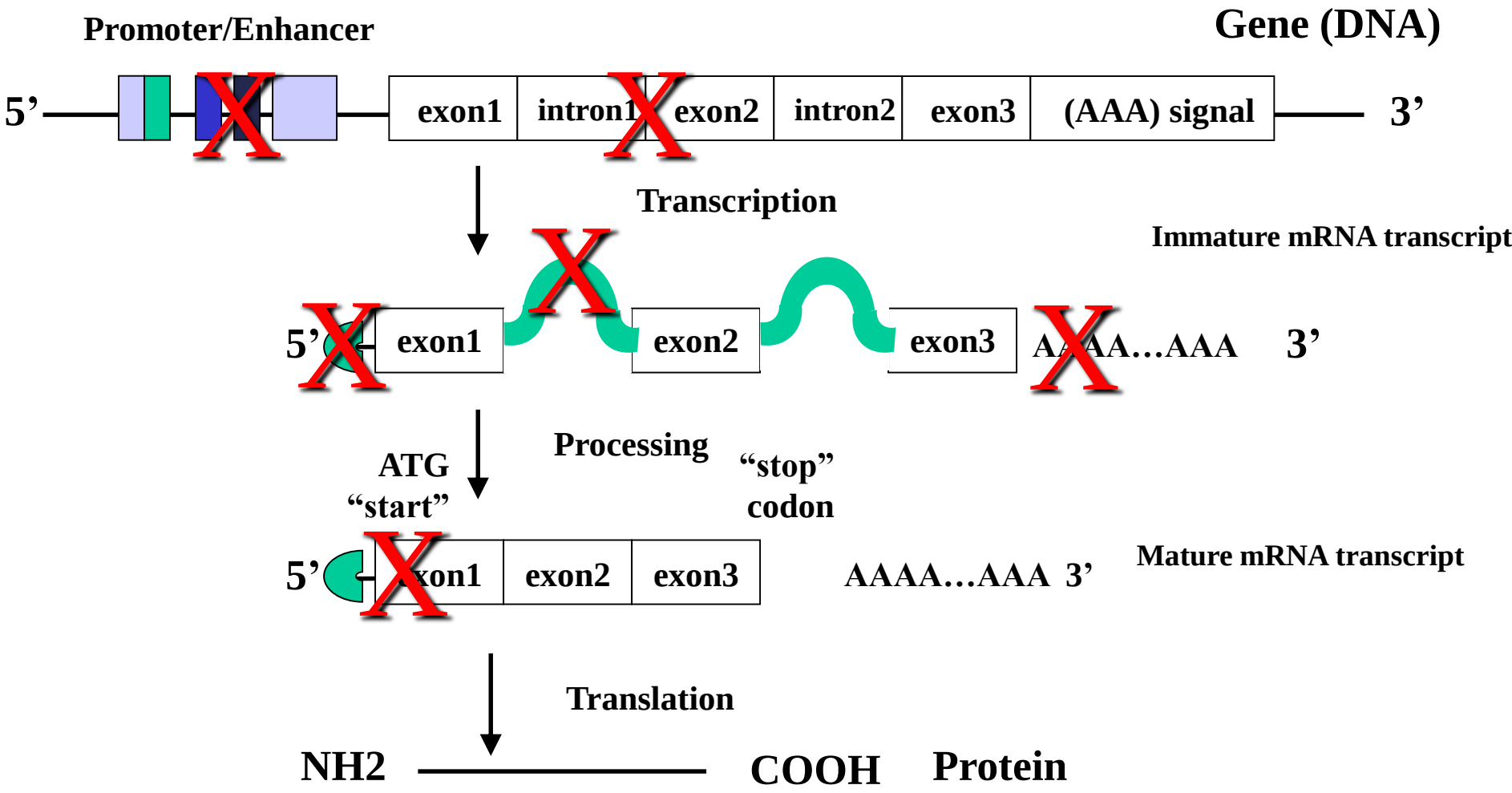
β Thalassemia

- They are the most important types of thalassemias because they are so common and usually produce severe anemia in their homozygous and compound heterozygous states (compound= when combined with other hemoglobinopathies or thalassemias)
- β thalassemias are autosomal inherited disorders of β globin synthesis. In most, globin structure is normal but the rate of production is reduced because of decrease in transcription of DNA, abnormal processing of pre-mRNA, or decreased translation of mRNA leading to decreased Hb-A production (A=Adult).

β Thalassemia

- Usually and mostly they are caused by gene mutations in the β gene in chromosome# 11, although deletions do occur.
- Hundreds of mutations possible in the β globin gene, therefore β thalassemia is more diverse disease in its presentation (the presentation differs between people depending on the type of mutation).
- This results in excess alpha chains, because they cannot find their counterparts (the beta chains) to bind to.

Type of mutations that could occur



Again:

- **β** thalassemias are usually and mostly due to single base pair substitutions rather than deletions. Although deletions do occur.

Beta (ß) thalassemia

- It appears when a person does not produce enough beta chains for hemoglobin.

- It is mainly prevalent in the Mediterranean region countries , such as Greece, Cyprus, Italy, Palestine and Lebanon.

ß thalassemia and malaria

- **Thalassemic RBCs offers protection against severe malaria caused by *Plasmodium falciparum.***
- **The effect is associated with reduced parasite multiplication within RBCs.**
- **Among the contributing factors may be the variable persistence of hemoglobin F, which is relatively resistant to digestion by malarial hemoglobinases.**

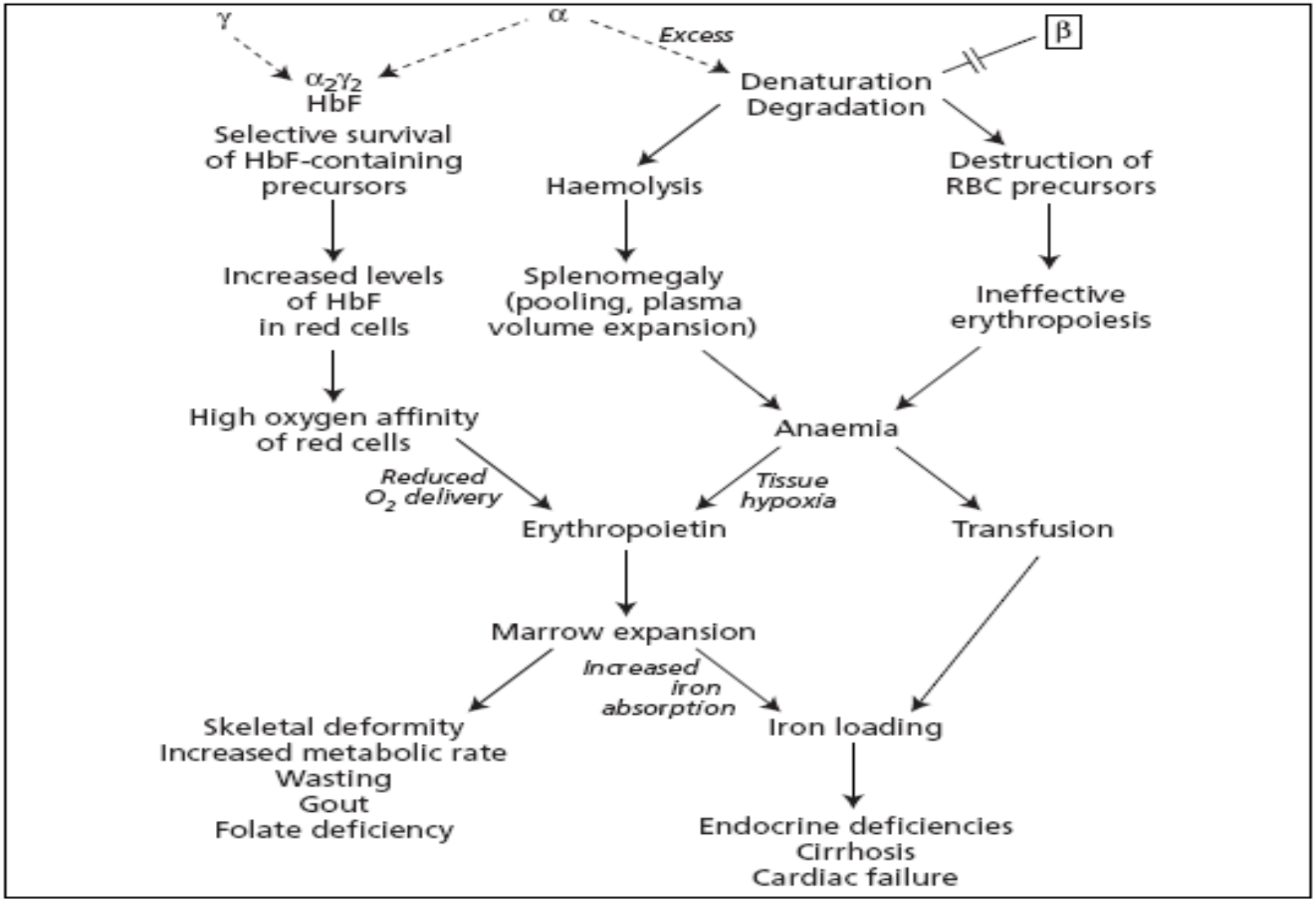
In brief:

- The anemia is due to two main components:
  - **Ineffective erythropoiesis (intramedullary).**
  - **Extravascular Hemolysis in RES esp. spleen**
- A third component that could contribute for the severity of anemia is **Splenomegaly** that may also worsen the anemia, because of two components: the (1) increased sequestration, and (2) increased plasma volume caused by the splenomegaly (dilutional).

There is also:

- Extramedullary erythropoiesis occurs, which also contributes for the splenomegaly, it is worthy to note that extramedullary erythropoiesis is not a perfect process, this is why in thalassemias we may see tear drop RBCs, and nucleated RBCs (NRBCs). Although, the NRBC seen in the blood film are from both the BM and the extramedullary erythropoiesis.

The pathophysiology of β-thalassaemia



At what age could β Thalassemia cause its effect???

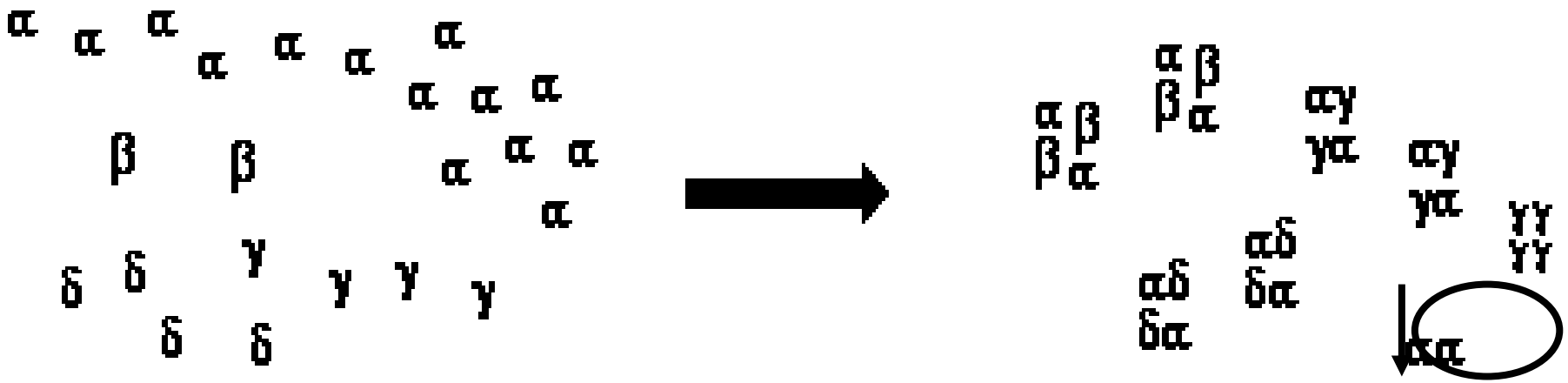
- In contrast to α globin, β globin is **not** necessary during fetal life (Hb-F= α2γ2), thus the onset of **β** Thalassemia isn't apparent until a few months after birth, when HbF is switched to HbA.

Types of βThalassemia

Three common types of β Thalassemia:

- β^{++} Thalassemia: The production of β chain is mildly reduced.
- β^{+} Thalassemia: The production of β chain is more reduced than β^{++} But NOT ABSENT. β^{++} and β^{+} are caused by mutation in Promoter region, 5`UTR, Cap site, Consensus sites, within Introns, 3`UTR, or Poly A site, and change in coding region.
- β^{0} Thalassemia: ABSENCE of β chain production. It is caused by mutation in Initiation codon, Splicing at junctions, Frameshift, Nonsense mutation.

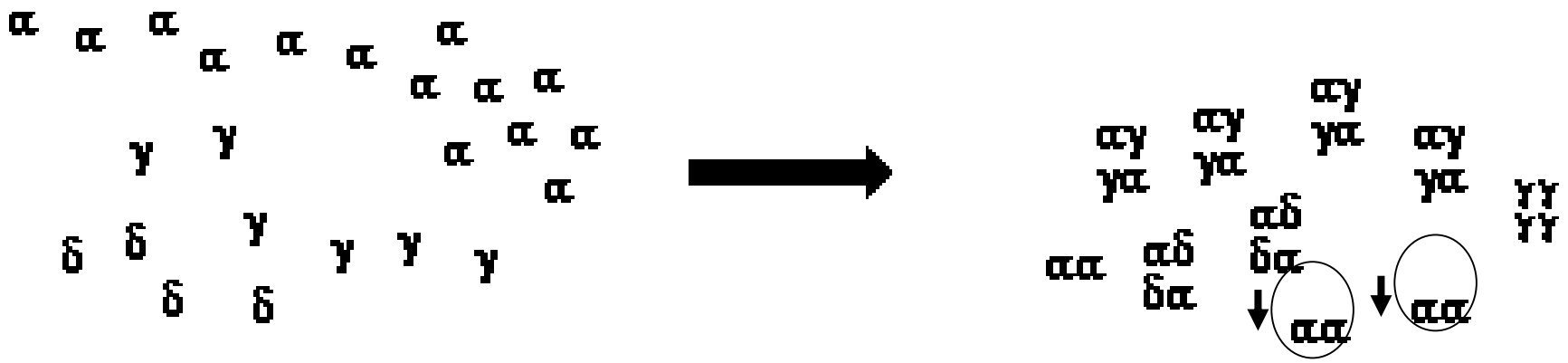
In β^{+} and β^{++} thalassemias, the mutated gene encodes for a small amounts of normal β mRNA. The quantity of β globin chain, which are made, varies largely from one molecular mutation/defect to another.



Excess α chains will precipitate in the RBC precursor cells and causes the ineffective erythropoiesis, also if it escape intramedullary ineffective erythropoiesis, RBCs possessing precipitated α chains will be hemolyzed in the P.B. by the RES (esp. in spleen).

β^0 Thalassemia

The β gene is unable to encode for any functional mRNA and therefore there is no β chain synthesis. So the situation will be more difficult than β^+ thalassemia.



More excess α chains will precipitate in RBC precursor cells and causes the ineffective erythropoiesis, also if it escape ineffective erythropoiesis, red cell possessing precipitated alpha chains will be hemolyzed in the P.B. by the RES (esp. in the spleen).

Again: What is Thalassemia?

- A group of inherited single gene disorders resulting in reduced or no production of one or more globin chains
- This results in an imbalance of globin chain production, with the normal excess chain producing the pathological effects:
  - Damage to RBC precursors →ineffective red cell production in BM.
  - Damage to mature red cells → hemolytic anemia
- Resulting in hypochromic, microcytic anemia

Each one of us inherit one gene from each parent

Homozygous: Normal Both gene are normal

β Gene

β Gene

Heterozygous: one normal and one abnormal/mutated

β Gene

β Gene

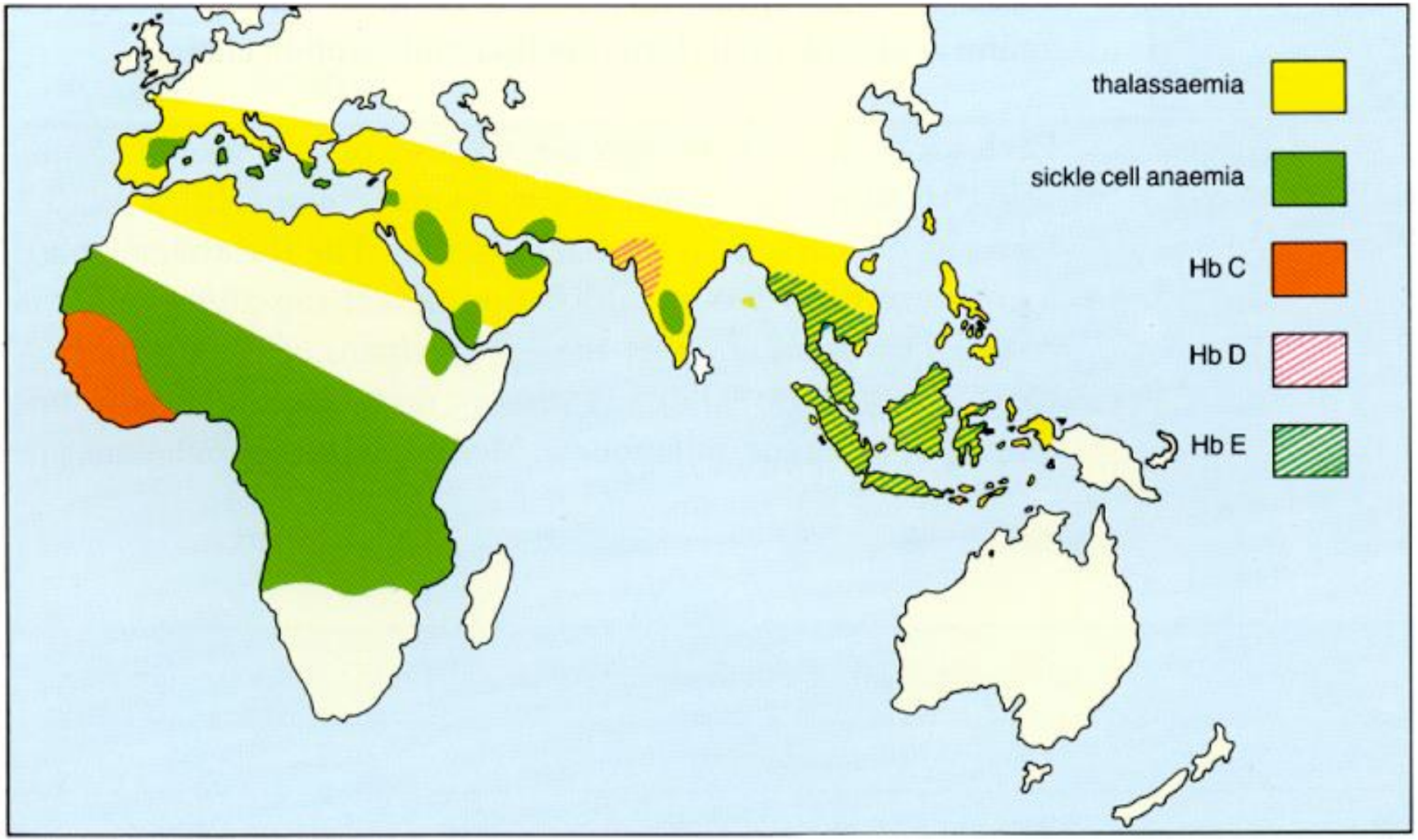
Homozygous: Abnormal Both gene are abnormal/mutated

β Gene

β Gene

Who is at risk?

Ethnic origin is very critical!



Classical Clinical Syndromes of β Thalassemia; β thalassemia can be presented as:

- Silent carrier state – mildest form of β thal.
- β thalassemia minor - heterozygous disorder resulting in mild hypochromic, microcytic hemolytic anemia.
- β thalassemia intermedia - Severity lies between the minor and major.
- β thalassemia major - homozygous disorder resulting in severe life long transfusion-dependent hemolytic anemia.

Silent Carrier State for β Thalassemia

- Are various heterozygous (from one parent) **β** gene mutations that produce only **small** decrease in production of **β** globin chains.
- Patients have nearly normal alpha/beta chain ratio and **no** hematologic abnormalities.
- Have normal levels of HbA_2.

β Thalassemia Minor (Trait)

- Caused by heterozygous (from one parent) mutations that affect β globin synthesis.
- β Chains production and thus Hb-A production is more reduced than the silent carrier Hb-A.
- Usually presents as mild, asymptomatic hemolytic anemia unless patient in under stress such as pregnancy, infection, or folic acid deficiency.
- Have one normal β gene and one mutated β gene.
- Hemoglobin level in 10-13 g/dL range with normal or slightly elevated RBC count (RCC).

β Thalassemia Minor (Trait)

- Anemia usually hypochromic and microcytic with slight aniso and poik, including target cells and elliptocytes; also may see basophilic stippling.
- Rarely see hepatomegaly or splenomegaly.
- Have high HbA_2 levels (3.6-8.0%) and normal to slightly elevated HbF levels.
- Normally require no treatment.

β Thalassemia Minor (Trait)

- 2- 6% HbF (N = < 1% after age 1 year)
- 3.6 - 8% HbA_2 (N = 2.2-3.6%)
- 87 - 95% HbA (N=95-100%)

β Thalassemia Intermedia

- Patients able to maintain minimum Hb (7 g/dL or greater) without transfusion dependence.
- Expression of disorder falls between thalassemia minor and thalassemia major.
- We will see increase in both HbA_2 production and HbF production.
- Peripheral blood smear picture is similar to thalassemia minor.

β Thalassemia Intermedia

- Have varying symptoms of anemia, jaundice, splenomegaly and hepatomegaly.
- Have significant increase in bilirubin levels.
- Anemia usually becomes worse with infections, pregnancy, or folic acid deficiency.
- May become transfusion dependent.
- Tend to develop iron overloads as result of increased gastrointestinal absorption.
- Usually survive into adulthood.

β Thalassemia Major

- Characterized by very severe microcytic, hypochromic anemia.
- Detected early in childhood:
- Hb level lies between 2 and 8 g/dL.
- Severe anemia causes marked bone changes due to expansion of marrow space for increased erythropoiesis (Epo is increased).
- See characteristic changes in skull, long bones, and hand bones.

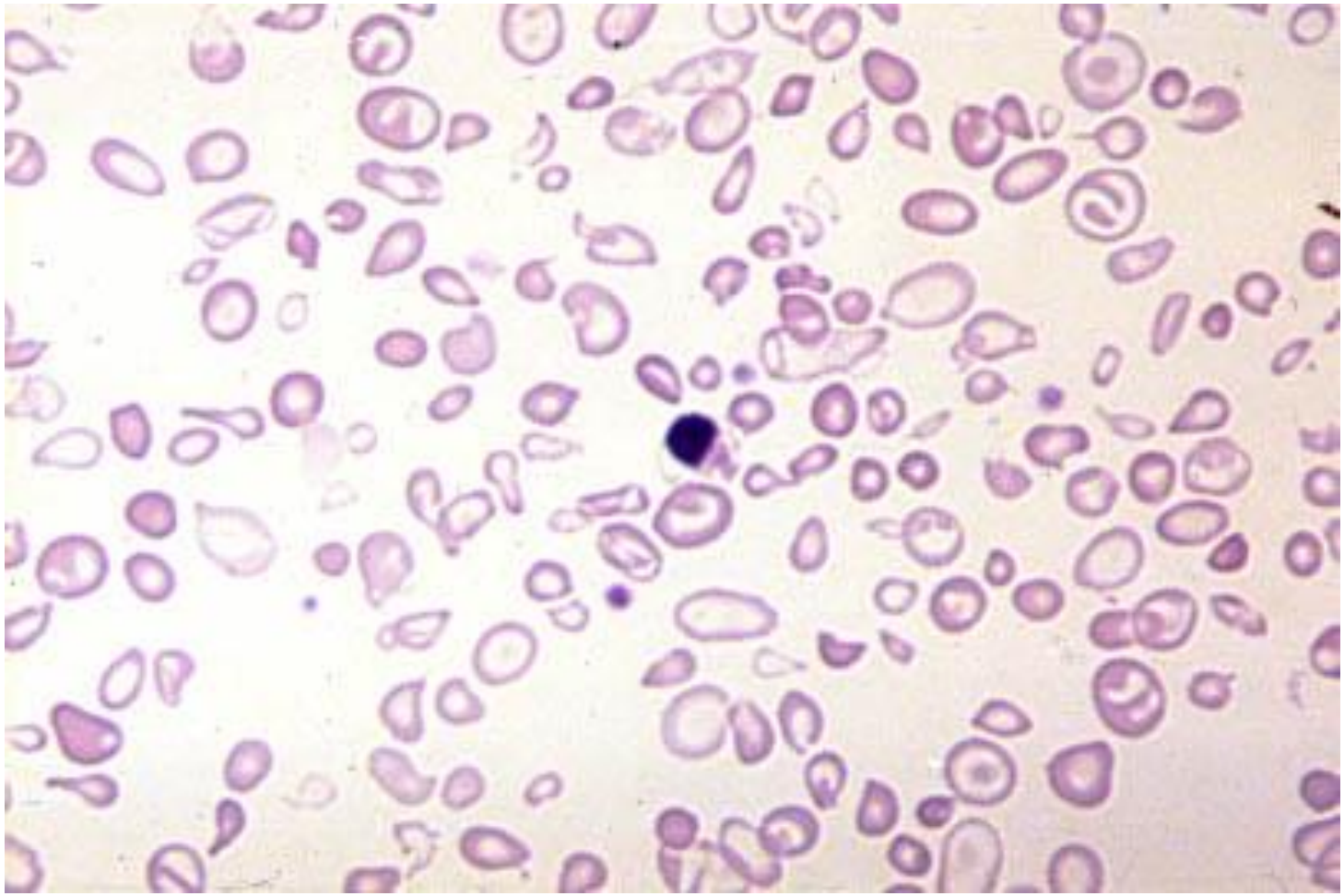
β Thalassemia Major

- Have protrusion upper teeth and Mongoloid facial features.
- Physical growth and development delayed.
- Peripheral blood shows markedly hypochromic, microcytic erythrocytes with extreme poikilocytosis, such as target cells, and elliptocytes. See marked basophilic stippling and numerous NRBCs.
- MCV in range of 50 to 60 fl.
- Retic count seen (2-8%).
- Most of Hemoglobin present is Hb F with slight increase in HbA_2.

β Thalassemia Major

- Regular transfusions usually begin around one year of age and continue throughout life.
- Excessive number of transfusions results in transfusional hemosiderosis; Without iron chelation, patient develops cardiac disease, liver cirrhosis, and endocrine deficiencies.
- Dangers in continuous transfusion therapy:
  - Development of iron overload.
  - Development of alloimmunization (developing antibodies to transfused RBCs).
  - Risk of transfusion-transmitted diseases (e.g. hepatitis, AIDS).
- Bone marrow transplants may be future treatment, along with genetic engineering and new drug therapies.

β Thalassemia Major



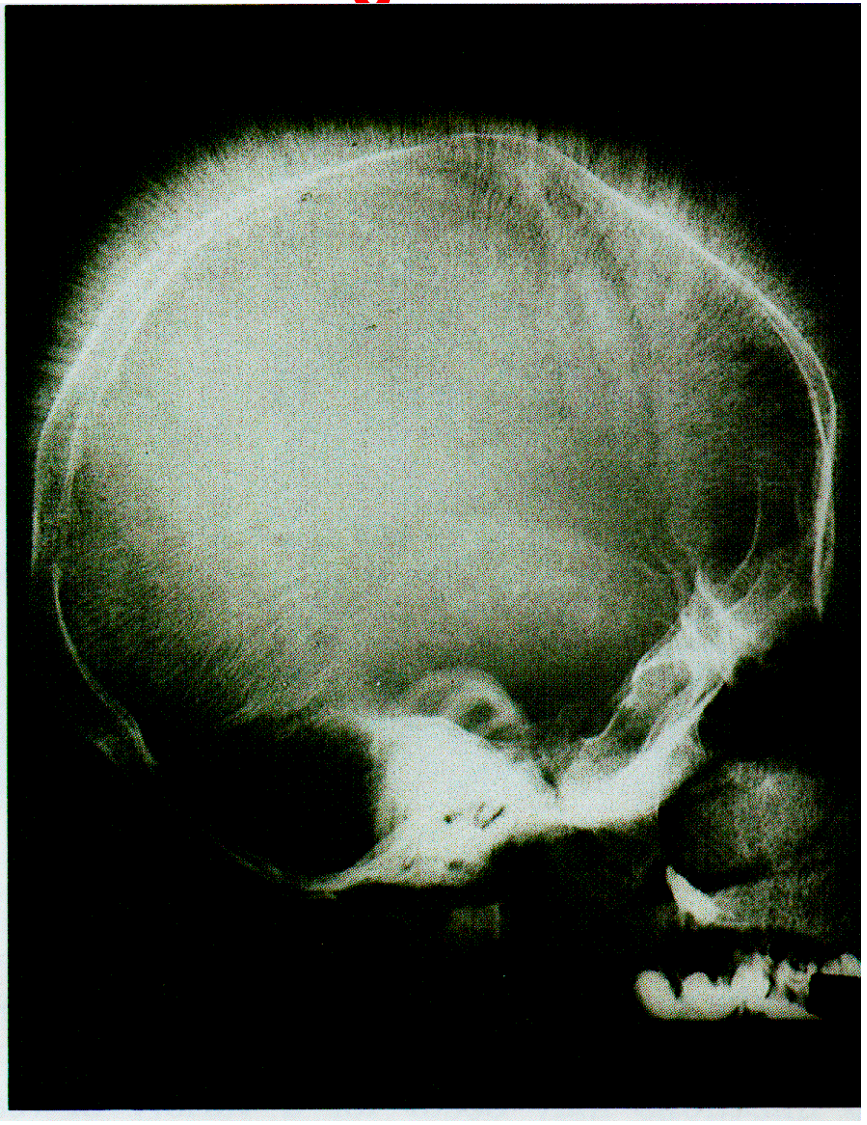
Anisopoikilocytosis, NRBC, microcytosis, hypochromia

Cooley's Anemia

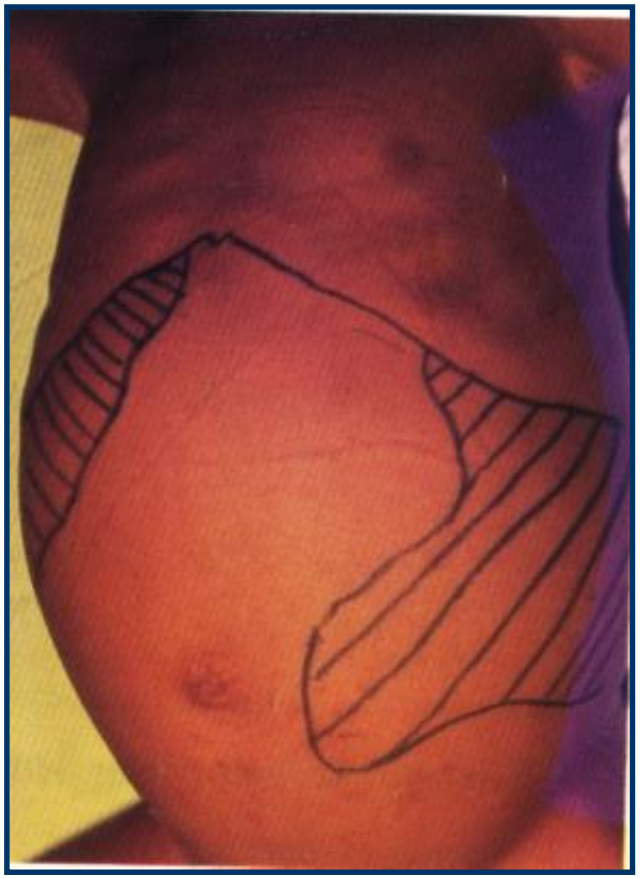
- This is another name for β Thalassemia Major, because Cooley was the first one to describe these cases.

Thalassemic face

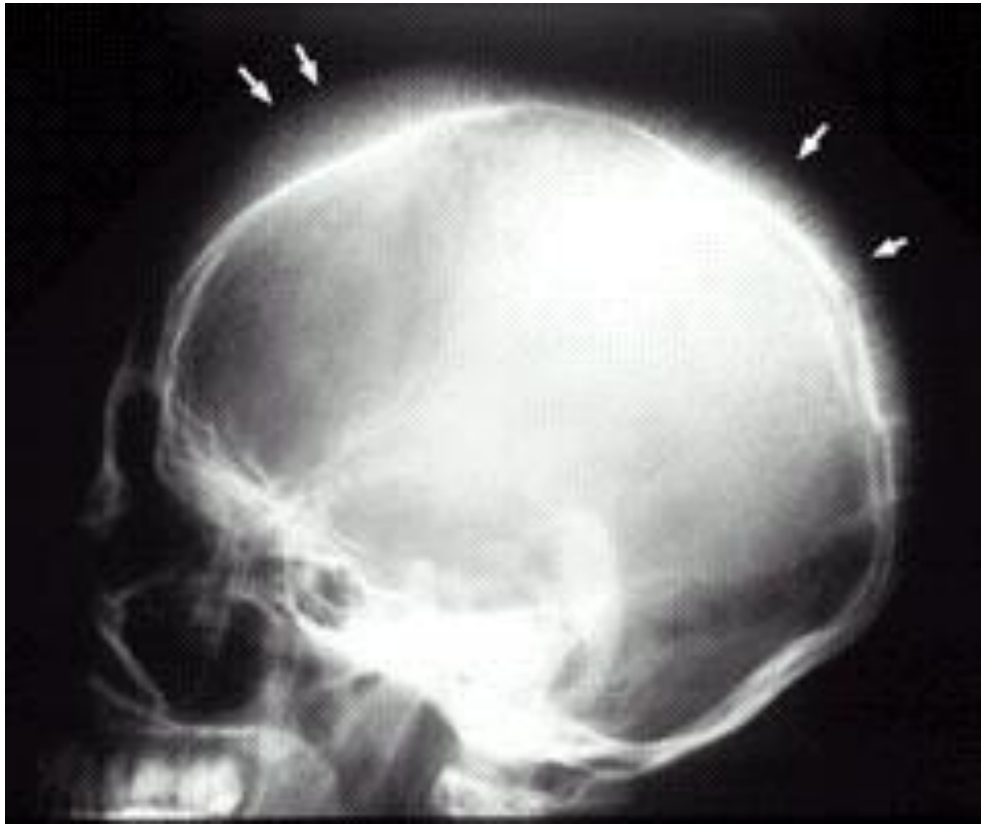
β Thalassemia Major



Hepatosplenomegaly



Hair on End Appearance



Dark skin due to iron overload

Thalassaemia major-life expectancy

- Without regular transfusion
  - Less than 10 years
- With regular transfusion and no or poor iron chelation
  - Less than 25 years
- With regular transfusion and good iron chelation
  - 40 years, or longer??

Good iron chelation using desferoxamine (iron chelator) prolongs the life expectancy of Cooley's anemic patients, otherwise cardiac failure, liver cirrhosis, and endocrine deficiencies could occur and causing death.

Comparison of β Thalassemias

GENOTYPE	Hb A	Hb A_2	Hb F
NORMAL	Normal	Normal	Normal
SILENT CARRIER	Normal	Normal	Normal
β THAL MINOR	Dec	N to Inc	N to Inc
β THAL INTERMEDIA	Dec	N to Inc	Increased
β THAL MAJOR	Dec	Usually Inc	Increased

δβ Thalassemia

- In some cases:
- They result from deletions of the δ and β globin genes.
- Homozygotes have 100% Hb-F, with moderate anemia 8-10 g/dl. With microcytosis and hypochromia.
- Heterozygotes have 15-25% Hb-F.

δβ Thalassemia

- They appear to have unequal crossing over between the δ and β globin gene loci with the production of δβ fused gene which codes for δβ fused globin chains that when combine to α chains forms an abnormal hemoglobin called Hb Lepore, which have an electrophoretic mobility like sickle hemoglobin.

δβ Thalassemia: Classification

- Because there are two causes for delta beta thalassemia: deletions and fusion. Delta Beta thalassemias can be classified into:
  - ($\delta\beta^0$) thalassemia: caused by complete deletion of δβ genes. Homozygotes characterized by 100% HbF. With moderate anemia 8-10 g/dl.
  - ($\delta\beta^+$) thalassemia: or called Hb Lepore thalassemia. Homozygotes have only HbF and Hb Lepore.

Hb A	$\alpha_2\beta_2$	96%
Hb A_2	$\alpha_2\delta_2$	3%
Hb F	$\alpha_2\gamma_2$	1%

Beta Thalassemia	Delta-Beta Thalassemia	Alpha Thalassemia
$\alpha_2\beta_2\downarrow$	$\alpha_2\beta_2\downarrow$	$\downarrow\alpha_2\beta_2$
$\alpha_2\delta_2$	$\alpha_2\delta_2\downarrow$	$\downarrow\alpha_2\delta_2$
$\alpha_2\gamma_2$	$\alpha_2\gamma_2$	$\downarrow\alpha_2\gamma_2$

Hemoglobinopathies

- Production of abnormal globin chains.
- Abnormal hemoglobins are the synthesised : structural variants.
- Abnormal hemoglobin has different property from its counterpart.
- Rate of synthesis of abnormal globin chain is reduced, resembling mild form of thalassemia

α–Structural Variants
(469 var submitted, July 2002)

- Hb Anantharaj cd11(Lys-Glu)
- Hb Mahidol cd74(Asp-His)
- Hb Siam cd15(Gly-Arg)
- Hb Suan Dok cd109(Leu-Arg)
- Hb Constant spring cd142(stop-Gln)

Hb Constant Spring

- Point mutation at termination codon
- **UAA-->CAA**
- 31 amino acids extension
- Total amino acid = 172
- Phenotype similar to α^{+}-thalassemia

β-Structural Variants

(649 var submitted, July 2002)

- Hb D-Punjab cd121(Glu-Gln)
- Hb J-Bangkok cd56(Gly-Asp)
- Hb S cd6(Glu-Val)
- Hb G-Siriraj cd7(Glu-Lys)
- Hb Tak cd147(+AC)
- Hb E cd26(Glu-Lys)

Hemoglobin E

- Commonly found in Thais
- Result of point mutation at codon 26 of b-globin gene.
- Glutamic acid-->Lysine
- Phenotype similar to β^{+}-thalassemia

Hemoglobin S

- Commonly found among the black.
- Cause of sickle cell anemia.
- Result of point mutation at codon 6 of β -globin gene; Glutamic acid-->Valine
- Hb S can perform polymerization, esp. when deoxygenated.

Order of Severity

- Hb Bart's hydrops fetalis.
- β-thalassemia major.
- β-thalassemia/Hb E disease.
- Hb H disease.

Clinical Symptoms

- Anemia, Jaundice.
- Hepatosplenomegaly.
- Bone change-->mongoloid face.
- Iron overload-->growth retardation, heart failure, DM, dark-colored skin , etc.

Management

- Blood transfusion
- Iron chelation: Desferroxamine,
- Splenectomy
- Stem cell transplantation : BM or Cord blood
- Prenatal diagnosis (PND)
- Supportive: Folic acid


THE END